Multi-trophic effects of invasive species suppression in a coastal wetland

by

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EXAMINING COMMITTEE MEMBERSHIP

The following served on the Examining Committee for this thesis. The decision of the Examining Committee is by majority vote.

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AUTHOR'S DECLARATION

This thesis consists of material all of which I authored or co-authored: see Statement of Contributions included in the thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

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STATEMENT OF CONTRIBUTIONS

I am the sole author of Chapters 1 and 5, which are not intended for publication. The three data chapters (Chapters 2 – 4) will be, or have been, published as stand-alone manuscripts. The work in Chapter 2 is in review as a manuscript co-authored by Dr. Rooney. I co-designed the study, conducted the field work, completed the data analysis, and wrote the resulting manuscript and Dr. Rooney co-designed the study, assisted with field work, data analysis, and authoring the manuscript. The work presented in Chapter 3 was completed as part of an on-going project led by the Ontario Ministry of Natural Resources and Forestry (OMNRF), in partnership with the Nature Conservancy of Canada (NCC), and the Ontario Ministry of Environment, Conservation and Parks (OMECP). This chapter is published in Invasive Plant Science and Management[1] with Dr. Rooney as a co-author. I was responsible for data collection, analysis, and authoring the manuscript while Dr. Rooney designed the monitoring approach, assisted with data analysis, and co-authored the manuscript.

Chapter 4 was co-authored by myself, Jacob Basso and Dr. Rebecca Rooney. For this chapter I designed the experimental approach, conducted the field work, assisted with laboratory work, conducted the statistical analyses, and authored the manuscript. Jacob Basso worked as a research technician with the macroinvertebrate project from 2018 – 2020 and was responsible for conducting the laboratory analysis (e.g., invertebrate identification, counting, weighing), assisting with field work as needed, and co-authored the manuscript. Dr. Rooney co-designed the experiment, assisted with statistical analyses, and co-authored the manuscript. As each data chapter is meant to stand alone as a research paper, I have maintained the use of "we" throughout to recognize the contributions of others to these projects.

[1] Robichaud, C.D. and Rooney, R.C. 2021. Suppression of established invasive *Phragmites australis* leads to secondary invasion in a coastal marsh. Invasive Plant Science and Management. https://doi.org/10.1017/inp.2021.2

ABSTRACT

Wetlands provide essential habitat for hundreds of species, including many that are of conservation concern in Canada. In southern Ontario, two wetland complexes, Long Point Peninsula and Rondeau Provincial Park, represent over 70% of the remaining intact wetlands on the north shore of Lake Erie. However, these wetlands are compromised by extensive invasion by non-native *Phragmites australis*. The conversion of these diverse wetlands into monocultures of an invasive species degrades wetland habitat and threatens species at risk. Ecological restoration presents a potential solution, but established invasive species can cause significant challenges to restoration. My research addresses key knowledge gaps regarding the success of *P. australis* in diverse wetland communities and how herbicide-based control of *P. australis* affects resident species in freshwater wetlands. The negative ecological impact of *P. australis* is evident by its rapid spread throughout North America and displacement of native plant species. However, there is a lack of field studies attempting to assess the role of fitness and niche differences (sensu Chesson 2000) in its success as an invader. To quantify niche and fitness difference between *P. australis* and resident wetland plants, I measured the performance of resident wetland plants with or without above-ground competition from *P. australis* and modeled the niche region of each species. My results indicated that *P. australis* intercepts more photosynthetically active radiation (PAR) than resident species, resulting in lower carbon assimilation rates for resident species growing with competition. In contrast, *P. australis* assimilates carbon more efficiently over the growing season and did not experience negative effects from competition with resident species. *Phragmites australis* also had the largest niche space of all four species, reflecting its wide range of environmental tolerances – in particular, soil moisture and nutrient levels - that have assisted its establishment throughout North America. Resident species had a high probability of overlap onto the niche space of *P. australis*, indicating it is excluding resident species from areas where they could otherwise persist. These results provide evidence that the ability of *P. australis* to reduce the availability of PAR for resident species and more efficiently assimilate carbon over the growing season, combined with high niche overlap, likely directly contributes to its success in North American freshwater wetlands.

Bringing this work into a management context, I measured the efficacy of herbicide control and assessed the recovery of native vegetation following the large-scale application of a

glyphosate-based herbicide directly over standing water to control *P. australis*. While herbicide was extremely effective at reducing populations of *P. australis* across a water depth gradient, passive restoration has resulted in a vegetation community dominated by non-native *Hydrocharis morsus-ranae* (European frogbit) at many treated sites. Since herbicide treatment began in 2016, Lake Erie water levels have been above average which contributed to the success of this free-floating aquatic invasive species. Finally, as drastic changes to vegetation will alter the way native biota use wetlands, I measured the macroinvertebrate community in sites invaded by *P. australis,* in herbicide-treated sites, and in remnant uninvaded marsh. Herbicide-treated sites had significantly higher macroinvertebrate densities and low taxonomic richness compared to *P. australis*-invaded sites and remnant marsh. Herbicide-treated sites had a macroinvertebrate community that was dominated by Chironomidae (Diptera). The sparse emergent vegetation, high water temperatures, and large amounts of decomposing biomass because of *P. australis* rolling and cutting in herbicide-treated sites likely favored these fast-growing detritivores that emerge from the water's surface. In contrast, taxa that required a platform (e.g., vegetation) from which to emerge were less likely to be present in herbicide-treated sites. These findings indicate that herbicide treatment in freshwater marsh dramatically changes the macroinvertebrate community composition. Future research should focus on incorporating fitness and niche differences into testing which native species may be able to coexist in a marsh where *P. australis* is being managed. Additionally, long-term research is required to assess how both native vegetation and macroinvertebrate communities recover from large-scale changes caused by invasive species control. These results together represent a comprehensive ecological assessment of the response of native biota to invasive *P. australis* and the large scale, herbicide-based control of *P. australis* in ecologically significant wetlands.

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DEDICATION

Dedicated to those I love who I lost along
the way: George, Carol, and Roy.

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1 General Introduction

1.1 Overview

More than half of all wetlands in the world have been lost (Zedler and Kercher 2005). In Canada, the Laurentian Great Lakes support diverse wetlands including over 268 km^2 of coastal wetlands on Lake Erie (Albert et al. 2005). Two large wetland complexes in particular, Long Point Peninsula and Rondeau Provincial Park, represent over 70% of the remaining intact wetlands on the north shore of Lake Erie (Ball et al. 2003). Rondeau Provincial Park and Long Point are designated "provincially significant wetlands" because of their biological, social, and hydrological value. This designation is necessary to protect these wetlands from development or alteration that could negatively impact the ecosystem services and essential habitat they provide. However, since the late 1990s both Rondeau and Long Point have experienced extensive invasion by non-native *Phragmites australis* subsp. *australis* ((Cav.) Trin. Ex Steud.) (e.g., Wilcox et al. 2003). The conversion of native, diverse vegetation communities into monocultures of an invasive species is a degradation of wetland habitat that provincial and federal legislation cannot easily prevent. Ecological restoration presents a potential solution, yet when an invasive species is entrenched in an ecosystem there are significant challenges to restoration.

1.2 Great Lakes coastal wetlands

1.2.1 Long Point and Rondeau wetland complexes

The marshes and swamps of Rondeau Provincial Park (9.3 km^2) are the largest wetlands in the central basin of Lake Erie while the wetlands of Long Point (134.65 km^2) are the largest wetlands in the eastern basin (Ball et al. 2003). Long Point, a 32 km sandspit, contains a mosaic of habitats. This includes complex wet sedge meadow, grass meadows (dominated by

Calamagrostis canadensis ((Mich.) P. Beauv.), and diverse emergent and aquatic communities containing rush, cattail, and various submerged and floating vegetation (Reznicek and Catling 1989) many of which are provincially rare plants (Ball et al. 2003, Prince et al. 1992; Reznicek and Catling 1989). Rondeau and Long Point support a similar diversity of plant species, though the wetlands in Long Point are more extensive than those in Rondeau (Reznicek and Catling 1989).

Long Point and Rondeau also provide essential habitat for species of invertebrates, herptiles, fish, birds, and mammals that rely on wetlands for a portion of their life cycle. Both wetlands are perhaps best known for the avifauna they support. Numerous bird species use the coastal wetlands of Long Point and Rondeau for migration and breeding, including species of conservation concern such as Least Bittern (*Ixobrychus exilis*), Virginia Rail (*Rallus limicola*), and Black Tern (*Chlidonias niger*) (Ball et al. 2003), and both wetlands represent some of the most important waterfowl habitat in the Great Lakes region (Knapton and Petrie 1999, Prince et al. 1992). The marshes also provide foraging habitat for numerous endangered species, including Barn Swallow (*Hirundo rustica*), an aerial insectivore that forages for invertebrates in flight (Meyer et al. 2010, Robichaud and Rooney 2017). Both Rondeau and Long Point are Important Bird Areas because of the globally significant waterfowl and migratory bird concentrations that use the marshes[2]. Long Point is also a designated UNESCO World Biosphere Reserve and a RAMSAR wetland of international significance.

[2] IBA Canada https://www.ibacanada.com

1.3 Invasive plant species

Precise terminology in invasion biology is crucial (e.g., Colautti and Macisaac 2004, Colautti and Richardson 2009, Davis 2011). For clarity throughout this document, I will be using the definition of "invasion" or "invasive" as defined in Richardson et al. (2000): an introduced species (one transported by humans across a major geographical barrier) that is able to overcome the abiotic and biotic limitations of a new region to create a self-sustaining population that spreads outside of the area where it was introduced. While this definition does not explicitly include ecological or economic impact, maybe invasive plant species can disrupt ecosystems and result in negative ecological impacts for native communities (Vilà et al. 2011). Invasive plants can have bottom-up effects within an ecosystem (Vilà et al. 2011) though competition with an invader rarely leads to the extinction of resident plant species (Sax and Gaines 2008).

While the consequences of invasive species can be serious, many introduced species fail to establish in a new environment (Mack et al. 2000, Leung et al. 2012). Many predictions derived from niche theory assume that a successful invasive species will gain access to untapped resources via unique traits, and that consequently a species-rich plant community should repel invaders as there are no "open niches" or unused resources (Elton 1958, Macarthur and Levins 1964, Levine and D'Antonio 1999). However, this often fails to predict which invasive species will have the largest negative ecological effects within a resident community (MacDougall et al. 2009). Therefore, MacDougall et al. (2009) proposed incorporating the concepts of "niche differences" and "fitness differences" from coexistence theory (Chesson 2000) to better predict which introduced species will be capable of both establishment and negative ecological impacts. In this framework, niche differences are defined as traits that have a stabilizing effect on

coexistence (e.g., density-dependent). A niche difference will provide an advantage when a species is rare, (e.g., a deep-rooted plant growing with shallow-rooted neighbours) but will limit that species via intraspecific competition as its population grows thus favoring coexistence (Chesson 2000). Fitness differences, however, will favour one competitor over another regardless of rarity in the community (e.g., density-independent) and, in the absence of niche difference, will result in the displacement of resident species (Chesson 2000, MacDougall et al. 2009).

Both niche and fitness differences have a strong influence on the outcome of community assembly (HilleRisLambers et al. 2012). In the context of invasions, niche differences increase the likelihood that an introduced species will be able to establish within a community, but without large fitness differences resident species may be able to coexist with the new species (Melbourne et al. 2007). Large fitness differences increase the probability of an introduced species having an *impact* on a resident community because they yield competition asymmetry (MacDougall et al. 2009). The asymmetry of competition will lead to one species exhibiting competitive dominance over another (Shea and Chesson 2002). If this competition asymmetry is combined with high niche overlap, and consequently that competition is also relatively intense, then the invasive species will most likely have a substantial negative ecological impact on resident communities (MacDougall et al. 2009).

1.4 Invasive *Phragmites australis* ssp. *australis*

Phragmites australis subsp. *australis* is a perennial wetland grass now present throughout much of North America (Catling and Mitrow 2011). The genus *Phragmites* currently includes four species of which only *Phragmites australis* has a global distribution (Saltonstall 2016). In

North America, invasive *P. australis* ssp. *australis* is considered a cryptic invader, as it resembles the native North American subspecies *P. australis* ssp. *americanus* (Saltonstall, P.M. Peterson, & Soreng) (Saltonstall 2002). Saltonstall (2002) determined that the *P. australis* that was rapidly expanding in North America was in fact not the native subspecies but rather the introduced *P. australis* ssp. *australis,* which is genetically closely related to *P. australis* in Europe, Asia, and Africa (Haplotype M) and thus constituted an invasive species. The first record of introduced *P. australis* ssp. *australis* in Canada is from 1910 in Nova Scotia, and by 1990 it had spread throughout the St. Lawrence river valley and Southern Ontario (Catling and Mitrow 2011) and was a dominant wetland species in many marshes (Lelong et al. 2007). *Phragmites australis* ssp. *australis* has continued to spread throughout Canada and is predicted to expand into the prairie provinces and much of southern Canada (Catling and Mitrow 2011). Populations of introduced *P. australis* were detected in Brooks, Alberta in 2016 and 2017 (Zuzak et al. 2018). For clarity in this document, any reference to *P. australis* will mean the introduced lineage, unless otherwise specified.

In the Great Lakes region *P. australis* populations are highest around Lakes Michigan, Huron, and Erie, all of which experienced water level decreases between 1999 and 2001 (Tulbure and Johnston 2010). The low water levels in Lake Erie during the mid-1980s likely provided a chance for *P. australis* to inoculate exposed soil, and to emerge during the next low nearly a decade later (Wilcox et al. 2003, Whyte et al. 2008, Tulbure and Johnston 2010). This is evident in Long Point, where *P. australis* is identifiable in aerial photos dating back to 1945 (4 ha to 17 ha) but began expanding exponentially in the late 1990s (from 18 ha to 137 ha in four years) (Wilcox et al. 2003). Ninety percent of the *P. australis* stands tested in Long Point around this time were the non-native haplotype M (Wilcox et al. 2003). Populations of *P. australis*

continue to grow at an annual rate of 14-37% in Long Point and are expected to continue at this rate into 2022 (Jung et al. 2017).

The negative ecological impacts of *P. australis* on wetland flora are extensive. *Phragmites australis* is highly productive, creating tall dense monocultures and extensive root and rhizome biomass (Packer et al. 2017). In mid-Atlantic brackish tidal marshes, *P. australis* populations grew rapidly until they reached a 50-80% occupation of the marsh (Lathrop et al. 2003). In the freshwater marshes of Long Point, *P. australis* has reduced the extent of native vegetation communities including meadow marsh, sedge/grass hummock, and emergent *Typha* marsh (Wilcox et al. 2003). Recent work indicates that low-lying marsh and shallow aquatic vegetation is currently the most vulnerable to *P. australis* invasion in Long Point (Jung et al. 2017). Comparisons of the effect of invasion between freshwater and brackish marshes determined that *P. australis* invasion decreases plant richness more drastically in freshwater marshes but is more likely to change the structure and function of less species-rich brackish marshes (Meyerson et al. 2000). The extensive above-ground biomass that *P. australis* creates also produces a considerable amount of litter which aids in the homogenization of wetland habitat structure (Able et al. 2003, Rooth et al. 2003). *Phragmites australis* invasion can rapidly convert resident vegetation communities into dense monocultures and alter the structure of wetlands.

While there is the potential for changes in wetland structure to affect the way fauna use wetlands, macroinvertebrates appear to be tolerant of *P. australis* invasion. In a drowned river mouth wetland on Lake Erie, macroinvertebrate densities were similar between *P. australis* and *Typha* (Kulesza et al. 2008). Whereas, on the south shore of Lake Erie, the densities of

macroinvertebrates using *P. australis* stands were higher than those in *Typha* stands (Holomuzki and Klarer 2010). Invertebrates (e.g., snails, amphipods, isopods) also had high abundances in *P. australis* stands in tidal marshes in the Connecticut River (Fell et al. 1998). However, *P. australis* invasion in a salt marsh located in New Jersey resulted in significant changes to the community structure of arthropods, including resulting in differences in spider guilds and supporting assemblages with more detritovores than native vegetation (Gratton and Denno 2005). This study by Gratton and Denno (2005) suggests that, while *P. australis* may support similar or even higher densities of invertebrates than the vegetation it displaces, it can cause important changes in community structure (i.e., Larsen et al. 2018).

Other wetland species have demonstrated negative responses to *P. australis* invasion. In freshwater marshes, endangered Blanding's turtle (*Emydoidea blandingii*) avoided patches of *P. australis*, effectively reducing the size of their home range, and nesting females that continue to move through *P. australis* stands risk desiccation (Markle and Chow-Fraser 2018). In Long Point, endangered Fowler's toad (*Anaxyrus fowleri*) populations started declining steadily in 2002, the timepoint when *P. australis* began to cover much of the marsh and converted shallow, sparsely vegetated breeding habitat into *P. australis* stands (Greenberg and Green 2013). And while *P. australis* patches are not ecological deserts in terms of the bird communities that use them (e.g., Gagnon Lupien et al. 2015), in Long Point bird species of conservation concern (e.g., Least Bittern, Virginia Rail) were not found using *P. australis* (Robichaud and Rooney 2017). As Long Point is a protected wetland in a highly developed landscape, the loss of its unique habitat to the unmitigated spread of *P. australis* could have devastating consequences for wetland species.

1.5 Ecological restoration

Ecological restoration is the process of assisting the recovery of an ecosystem that has been degraded, damaged, or destroyed (SER 2004). The substantial loss of wetlands globally, and the important ecosystem services they provide, makes wetland restoration an important mitigation tool that can reduce the impact of wetland degradation or loss (Zedler and Kercher 2005). The marshes of Long Point and Rondeau exist within a landscape that has otherwise been converted to agriculture or other forms of development. Southern Ontario has lost approximately 72% of its wetlands since settlers arrived (circa 1800), with a continued loss of an estimated 3,543 ha per year since 1982, despite a prioritization of wetland protection and restoration (Ducks Unlimited Canada 2010). Restoration of Great Lakes coastal wetlands can range from altering the physical environment to encourage the return of natural hydrology (e.g., dikes, dams, or improved hydrological connections) or manipulating biological communities by boosting the populations of desired species or controlling unwanted, non-native species (Wilcox and Whillans 1999). In the case of the wetlands in Long Point and Rondeau, the continued growth of *P. australis* populations and loss of habitat for species-at-risk constituted a high priority concern for land managers. Ecological restoration of these wetlands, which began in earnest in 2016, is focused on the control or removal of *P. australis* with the intention of catalyzing the recovery of native vegetation and improving habitat for species-at-risk (specific project details in Chapter 3).

Invasive species pose a considerable challenge in ecological restoration. Changes to ecosystems because of invasion, such as nutrient cycling, can create a positive feedback that entrenches the invasive species and may limit native species recovery (Yelenik and D'Antonio 2013). It is possible that ecosystem changes reinforce invasive populations and represent an

alternative stable state in wetland communities that will return in the absence of continued manipulation (e.g., control action) (Beisner et al. 2003). Even if a control project is able to reduce or eradicate an invasive species population there will often be legacy effects within the system (D'Antonio and Meyerson 2002). This can include a seed bank with fewer native species (Gioria et al. 2014), one that contains viable seeds of the recently removed invasive species (e.g., Howell 2017), or a thick litter layer that can inhibit native plant germination (Kettenring and Tarsa 2020). The survival of seedlings was reduced when growing in soil conditioned by both non-native and native *P. australis,* indicating that there are likely legacy soil effects that limit recovery after non-native *P. australis* is removed from wetlands (Crocker et al. 2017). The legacy effects left behind by invasive species can favour re-invasion of the target species or secondary invasions by different non-native plants (Hess et al. 2019). A meta-analysis determined that targeted invader suppression is a key factor in driving secondary invasions (Pearson et al. 2016). These factors can jeopordize the success of a restoration project by limiting the recovery of native vegetation. Additionally, the long-term continued control necessary to keep invasive species populations at a low level (see next section) are more likely to have a negative effect on native species (Kettenring and Reinhardt Adams 2011).

1.6 *Phragmites australis* control options

Phragmites australis control can involve multiple approaches including mechanical control (mowing, cutting, burning), biological control (e.g., herbivory, biocontrol, plant competition) or chemical control (herbicide application) (Hazelton et al. 2014). Mechanical control options do little on their own to reduce the dominance of *P. australis* and some approaches, like mowing, can increase shoot production (Derr et al. 2008, Hazelton et al. 2014).

In addition to their relatively low efficacy, mechanical removal can be labour intensive and is not always feasible when the targeted *P. australis* population is very large or spans a challenging landscape. However, when combined with herbicide-based control mechanical methods play an important role in removing standing *P. australis* stems (e.g., knocking them down, burning) or improving herbicide efficacy (Lombard et al. 2012).

Biological control offers an approach that could suppress *P. australis* populations at a landscape level without the need for heavy machinery or herbicide use. There has been demonstrated public interest in biocontrol, with 91% of land managers indicating they would be comfortable using biocontrol if agents demonstrated no risk to native *P. australis* (Martin and Blossey 2013). As the invasive strain of *P. australis* is phylogenetically distinct from the native strain, researchers have been able to identify two candidate moth species (*Lenisa geminipuncta* and *Archanara neurica*) that are highly specific to introduced *P. australis* (Blossey and Casagrande 2016). The timelines for widely available biocontrol are still unclear and debates in the literature are ongoing (e.g., Blossey et al. 2020), despite evidence suggesting biocontrol is both safe and feasible. Promisingly, a team led by researchers at the University of Toronto and Agriculture and Agri-Food Canada have applied for open field release permits, so this additional *P. australis* management tool may soon be available in Canada (McTavish et al. 2020).

Most *P. australis* control projects use herbicide or a combination of herbicide and mechanical control (Kettenring and Reinhardt Adams 2011, Hazelton et al. 2014). Indeed, a 2014 review found that 94% of US organizations used herbicide (either glyphosate or imazapyr) as their primary control method (Martin and Blossey 2013). Both imazapyr and glyphosate are non-selective herbicides, meaning they will kill any plants to which they are applied, so

practitioners must be careful to apply it to only targeted *P. australis.* The active ingredients imazapyr and glyphosate have been deemed nontoxic to animals on their own, thus multiple formulations for application over standing water are available in the U.S. (Hazelton et al. 2014). In *P. australis* control projects in the U.S., glyphosate is the most used active ingredient and is typically applied at the end of the growing season after native plants have senesced (Kettenring and Reinhardt Adams 2011, Hazelton et al. 2014). Until recently in Canada, any available imazapyr- and glyphosate-based herbicides were only registered for terrestrial use as their formulations contain surfactants that are acutely toxic to aquatic biota, such as amphibians (e.g., Relyea 2005, Mesnage et al. 2019). While these products are useful for controlling *P. australis* in dry habitats, the lack of an appropriate herbicide to use in areas with standing water has limited control of this wetland grass. The *P. australis* control project reported on in Chapter 3 and 4 occurred after the Ontario Ministry of Natural Resources and Forestry and partners obtained an Emergency Use Registration (#32,356) under the Pest Control Products Act from Health Canada's Pest Management Regulation Authority and a provincial Permit to Perform an Aquatic Extermination. This allowed them to apply a glyphosate-based herbicide (Roundup R Custom for Aquatic & Terrestrial Use Liquid Herbicide, Registration Number 32,356 Pest Control Products Act) combined with a non-ionic alcohol ethoxylate surfactant (Aquasurf ®, Registration Number 32,152) to control invasive *P. australis* in standing water (Robichaud and Rooney 2021). Recently in Canada (March 2021), an imazapyr-based herbicide (Habitat® Aqua) manufactured by BASF Canada Inc. (Mississauga, Ontario) was registered for use in areas with standing water and can be applied by practitioners with aquatic pesticide licenses. In Ontario, best practices for *P. australis* control are a combination of herbicide application, where allowed, and secondary mechanical treatment to remove standing dead biomass (OMNRF 2011).

Despite the wide-spread use of herbicides to control *P. australis* there is little quantitative evidence that herbicide application leads to *P. australis* eradication. In interdunal wetland swales, 14 ha of *P. australis* were treated by cutting the plant and applying a glyphosate-based herbicide to the stem (Lombard et al. 2012). Continual treatment resulted in the steady decline of *P. australis* populations, with the cost of maintenance declining in parallel, though *P. australis* was not completely eradicated after seven years (Lombard et al. 2012). An assessment of outcomes after treating over 300 patches of *P. australis* with glyphosate-based herbicide determined that the probability of eradicating *P. australis* (no growth for three consecutive years) decreased as patches got larger – the probability of eradication at a medium patch (45 m^2) was 0.26 and at a large patch (> 3000 m^2) it was 0.02 (Quirion et al. 2017). Patch scale was also an important determinant in work conducted in wetlands on the Great Salt Lake (Rohal et al. 2019). *Phragmites australis* was more likely to re-invade large patches (12,000 m^2) two years after treatment (glyphosate or imazapyr herbicide & winter mowing), possibly driven by the prolonged flooding and hydrological disturbance in these sites relative to smaller patches (Rohal et al. 2019). The results of this work suggest that *P. australis* management should focus on smaller patches with less hydrological disturbance that are adjacent to native vegetation, as this will minimize the amount of *P. australis* regrowth and maximize native vegetation recovery (Rohal et al. 2019). These results emphasize that the environmental conditions of a site can have a strong influence on the trajectory of *P. australis* control and native vegetation recovery. As shown with the above examples, herbicide requires continued applications to maintain *P. australis* population suppression. Yet, glyphosate-based herbicides have come under increased scrutiny recently because of their alleged health effects and their ubiquitous application (Myers

et al. 2016) and we lack information on the long-term consequences of its widespread and repeated use on wetland ecosystems (Blossey and Casagrande 2016).

To responsibly manage biological invasions, the effects of large-scale control must be carefully weighed against the effects of invasion (Kopf et al. 2017). This approach should aim to probabilistically assess the risks of the continued spread of the invader, the control options available, and the probability of success (Hulme 2006). It is often assumed in wetland restoration that if the hydrology is appropriate, then native species will recolonize a degraded area (Palmer et al. 2017). This assumption needs to be carefully evaluated through effective monitoring, especially in the face of invasive species removal.

1.7 Research objectives

Restoration projects with the goal of removing an established invasive species present challenges for practitioners. These challenges are often compounded by a lack of comprehensive ecological monitoring that can identify 1) the impact of an invasive species, 2) the impact of invasive species control and/or restoration actions, and 3) the recovery of native biological communities. My thesis addresses these gaps with glyphosate-based *P. australis* control and passive restoration of wetland vegetation in coastal marsh of the Rondeau Provincial Park and the Long Point Peninsula. In my second chapter, applying concepts from coexistence theory (e.g., Chesson 2000, MacDougall et al. 2009), I conduct a field experiment to assess the effect that competition for photosynthetically active radation with *P. australis* has on resident wetland species and determine the niche overlap among species based on light availabiltiy and soil conditions. While examining only a few axes of each species' niche, this work provides insight into what mechanisms allow *P. australis* to so successfully establish in North American

freshwater marshes. Further, it provides evidence that niche preemption by dominant resident flora is not enough to prevent invasive *P. australis* establishment or its re-establishment following herbicide treatment. In my third chapter, I assess the efficacy of the first large-scale (>400 ha) glyphosate-based herbicide control of *P. australis* in Canada. Working closely with the Ontario Ministry of Natural Resoruces and Forestry, the Nature Conservancy of Canada, and other partners, I assessed how effective herbicide-based control was at preventing *P. australis* re-growth along a water depth gradient and which plant species colonized treated areas one- and two- years after treatment using a Before-After-Control-Impact approach. In my fourth chapter, I compare the macroinvertebrate communities present in *P. australis* stands, remnant uninvaded marsh habitat, and herbicide-treated sites in Long Point. To capture the diversity of macroinvertebrate assemblages in the marsh, I compare aquatic macroinvertebrates taken from submersed aquatic vegetation and emerging macroinvertebrates taken from emergence traps. Together these two chapters represent comprehensive ecological monitoring necessary to assess the response of biological communities to the large-scale control of *P. australis.* In my final chapter I integrate findings from all three chapters, summarize the management implications of glyphosate-based herbicide *P. australis* control, and suggets avenues of future research.

2 An assessment of resource acquisition and niche overlap between non-native *Phragmites australis* and resident plant species in a freshwater wetland.

2.1 Introduction

Identifying the mechanisms that confer an advantage to an introduced species can be challenging. Work rooted in classic niche theory suggests that a resident community may have niche space, or untapped resources, that can be exploited by an introduced species (e.g., Elton 1958, Richardson and Pyšek 2006). It is also often posited that a successful invader is a superior competitior compared to its native neighbours, though this advantage can be transient or might works in concert with other environmental variables (Gioria & Osborne, 2014). For example, the interactions between environmental and biological components of a system will influence community composition and species establishment (e.g., Kraft et al., 2015), while fluctuations in spatial or temporal niche opportunities (e.g., Chesson & Huntly, 1997) can favour the coexistence of seemingly similar species. The complexities of biological communities can make it difficult to predict the effects of invasive species using a unified framework.

Both community assembly (Pearson et al. 2018) and coexistence theory (MacDougall et al. 2009) have provided structure to guide invasion ecology that account for the complexities of ecological interactions. In the community assembly framework, invasive species might "break the rules" of the resident community by having rare traits that benefit them relative to community-specific conditions (Pearson et al. 2018), for example, traits that allow them to better take up resources or cope with reduced resources (Gioria and Osborne 2014). MacDougall et al. (2009) applied a coexistence theory (*sensu* Chesson 2000) framework to invasion ecology and

proposed that niche differences (or differences that benefit a species when they are rare) and fitness differences (or differences that benefit one species regardless of their rarity in the community), jointly explain how introduced species become established and why they are deleterious to the resident community. Essentially, an introduced species with a large fitness difference and high niche overlap with the resident community is more efficient at drawing down shared resources and therefore more likely to establish and result in negative ecological impacts, such as displacing native plant species (MacDougall et al. 2009).

Phragmites australis subsp. *australis* (European common reed) is a perennial wetland grass originally from Europe that is now found throughout North American wetlands (Saltonstall 2002). Compared to the North American native *Phragmites australis* subsp. *americanus,* invasive *P. australis* generates more above-ground biomass, higher specific leaf area, and has a higher relative growth rate (Mozdzer and Megonigal 2012). Additionally, invasive *P. australis* tolerates a wide range of environmental conditions. These includes surviving in water depths ranging from -30 cm to 70 cm above ground-level (Haslam 1971), in freshwater and salt marshes (e.g., Konisky and Burdick 2004, Vasquez et al. 2005), and in a wide range of soil nutrient concentrations (Packer et al. 2017) – Meyerson et al. (1999) reported *P. australis* growing in soil with a range of 0.35 – 14.7 mg g^{-1} nitrogen. To accommodate these varying environments, invasive *P. australis* exhibits phenotypic plasticity (e.g., Richards et al. 2006) – for example, invasive *P. australis* can adjust its ratio of root:shoot biomass in response to nutrient conditions (Minchinton and Bertness 2003). Invasive *P. australis* also responds positively to the clearing of above-ground biomass and nutrient addition by increasing the density and height of above-ground shoots (Minchinton and Bertness 2003) and generates more total biomass when grown in elevated CO_2 treatments, suggesting it may experience increased relative fitness as CO_2 levels

continue to rise (Mozdzer and Megonigal 2012). Due to the extensive amount of research conducted on invasive *P. australis,* it has been formally suggested as a model organism with which to study plant invasions (Meyerson et al. 2016).

Invasive *P. australis* presents an interesting case of an invasive species that can establish itself in highly productive wetland ecosystems where niche space should be scarce (e.g., Macarthur and Levins 1964, Levine and D'Antonio 1999, Keane et al. 2002). In saltmarshes (e.g., Chambers et al. 1999) and freshwater marshes (e.g., Wilcox 2012) of North America, invasive *P. australis* has replaced native vegetation communities and reduced plant species richness (Meyerson et al. 2000). The species-rich plant communities located in the coastal marshes of Long Point, a peninsula on the north shore of Lake Erie, are one such system that has been largely converted to invasive *P. australis* (haplotype M) in the last few decades (Wilcox et al. 2003). Beginning in the mid-1990s invasive *P. australis* cover increased in Long Point (Jung et al. 2017), increasing in aerial extent exponentially from approximately 18 ha in 1994 to 137 ha in 1999 (Wilcox et al. 2003). During this period of growth *P. australis* most often replaced *Typha* spp., meadow marsh, and sedge or grass hummock species (Wilcox et al. 2003). More recent work suggests that if water levels remain stable *P. australis* will continue to spread at current rates (14 - 37% annually), with marsh and shallow aquatic vegetation vulnerable to replacement (Jung et al. 2017).

While there is clear evidence that *P. australis* is successfully displacing resident plant communities in freshwater marshes, there remains a dearth of field studies that quantify niche overlap and fitness difference between *P. australis* and resident species. The marshes of Long Point provide an opportunity to address this knowledge gap. We hypothesize 1) that *P. australis*

will more efficiently assimilate carbon than resident species, a fitness difference that contributes to its success in diverse vegetation communities, 2) that *P. australis* will intercept more photosynthetically active radiation than resident species and lead to a negative effect of competition for resident species, and 3) that *P. australis* will have considerable overlap with the niche space of resident species due to its wide range of environmental tolerances. This experiment provides an opportunity to apply the coexistence framework suggested by MacDougall et al. (2009) to a well studied invasive species and explain a component of the mechanisms that make *P. australis* such a detrimental invasive species.

2.2 Methods

2.2.1 Study site

Our study took place in Long Point Provincial Park (Appendix 1A), Long Point, Ontario, CA (42°35`01" N, 80 °22` 37" W). The resident vegetation communities in our study marsh assemble along a moisture gradient from deeper standing water (approximately 20 – 70 cm) to shallow standing water or saturated soils (approximately 0 – 30 cm). Hybrid *Typha* x *glauca* (Godr. (pro sp.)), an invasive cattail resulting from hybridization between *Typha latifolia* (L.) and *Typha angustifolia* (L.) (Pieper et al. 2020), are dominant in the emergent marsh that is characterized by deeper standing water. On the opposite end of the moisture gradient is meadow marsh containing a diverse vegetation community composed of grasses (e.g., *Calamagrostis canadensis* ((Michx.) P. Beauv.)), sedges (e.g., *Carex* spp.), rushes, and forbs. Both vegetation communities have been displaced by *P. australis* over the last two decades (Wilcox et al. 2003).

2.2.2 Phytometer measurements

For our experiment we selected phytometers (an individual stem/ramet used to measure plant responses to experimental manipulations) of *P. australis* and three species that were most abundant in the remnant vegetation communities. For emergent marsh, we chose *Typha* spp. (cattail) as the phytometer species. These phytometers are likely *Typha* x *glauca*, but as identification based on morphology is challenging due to the amount of hybridization in the field (Travis et al. 2010, Bansal et al. 2019). We refer to these phytometers as "*Typha* spp." throughout. For meadow marsh, *Carex aquatilis* (water sedge (Wahlenb.)) and *Calamagrostis canadensis* (Canada bluejoint grass) were chosen as representative species. Hereafter, *Typha* spp., *C. aquatilis*, and *C. canadensis* are referred to as "resident species". Each resident species is a densely growing rhizomatous perennial that reproduces sexually and asexually, traits that are shared with *P. australis*. Additionally, *Typha* x *glauca* generates dense monocultures (e.g., Galatowitsch et al. 1999) like invasive *P. australis*, and *C. canadensis* is taxonomically closely related to *P. australis* as they both belong to the Poaceae family.

Each phytometer was randomly assigned to either grow with above-ground competition or without above-ground competition in the field. Phytometers of resident species grew with competition from *P. australis*, while *P. australis* phytometers grew with competition from *Typha* spp., *C. canadensis*, or *C. aquatilis*. All phytometers were situated along the leading edge of a *P. austral*is stand. This best approximates realistic competition in invaded areas, as *P. australis* relies mostly on clonal expansion once it has established itself through seeds or clonal propagules (Kettenring et al. 2016). To minimize intraspecific competition, we selected resident phytometers that were growing surrounded primarily by *P. australis* within the stand and *P. australis* phytometers that were growing surrounded by each resident species. All the

phytometers were established in the same area, with a maximum distance of 150 m between plots (Appendix A).

We established 96 individual phytometers on 23-May-2016 (Appendix 1B) and 27-May-2017 (Appendix 1C), for a total of 192 phytometers. In both years, we established 24 phytometers (12 with competition, 12 without competition) of both *Carex aquatilis* and *C. canadensis*. We established 12 phytometers (6 with competition, 6 without) of *Typha* spp., and *P. australis* and its respective neighbours (12 x 3 = 36), as these species were less likely to be damaged over the field season. During set up, each phytometer was paired by height between treatments to ensure that the full range of heights in the early growing season were represented and that plant sizes covered a similar range in both treatments (Appendix 1D). We cleared all standing above-ground biomass in the 'without competition' treatments by clipping the 1 m^2 area surrounding the phytometer. We did not alter the surrounding aboveground biomass within 1 m^2 around the phytometer in the "with competition" treatment (Appendix 1E). However, as all the phytometer species are clonal, we severed roots and rhizomes connecting phytometers to surrounding plants in both the 'with competition' and 'without competition' treatments by sawing the perimeter of the 1 m^2 plots to a depth of 50 cm with a hand saw in May. We performed this once, as pilot work determined that severing the below-ground material more than once over the growing season resulted in too much physical disturbance to the plot. Phytometers of *C. aquatilis* and *C. canadensis* were supported with a bamboo stake, as pilot work determined that they required extra support to prevent tissue damage from hydrodynamics and wind action once the surrounding vegetation was removed. Over the course of the experiment all phytometers were subject to stress, and those that were consumed or died and are reported in Appendix 1B & 1C.

Once plants had matured, we measured the carbon assimilation rate (μmol CO_2 s^{-1} m^{-2}) (A) and photosynthetic water use efficiency (CO_2 mmol s^{-1} m^{-2} H_20) (WUE) of each phytometer using a CIRAS-3 true differential gas analyzer with a PLC3 Universal LED Light Unit (RGBW) and PLC3 narrow leaf cuvette (PP Systems, Amesbury, MA, USA). We selected a fresh, entire (e.g., no damage) leaf growing in full sunlight from each phytometer and then measured a photosynthesis-irradiance (PI) curve in the field. The PI curve began by exposing the leaf to 1500 μmol s^{-1} m^{-2} of photosynthetically active radiation, equivalent to an average full-sunlight day during the growing season, and slowly reduced PAR to 1000, 500, 200, 100, 50, and 0 μmol s^{-1} m^{-2} while simultaneously measuring carbon assimilation rate and photosynthetic water use efficiency. Measurements at each PAR level were taken until carbon assimilation rates plateaued, which typically occurred within two to three minutes. We took these measurements from July 26th to August 2nd in 2016 and from July 4th to July 14th in 2017, with phytometers of the same species measured on the same day to reduce potential temporal differences in performance between treatments. We also measured the amount of PAR reaching the top of each phytometer relative to the incident PAR above the canopy using a LI-1500 Light Sensor Logger coupled with two LI-190R quantum sensors (Li-Cor Biosciences, Lincoln, NE, USA). These sensors were deployed to take simultaneous readings from above the canopy and the phytometer to most accurately calculate the percent of light intercepted by the canopy. PAR measurements were taken on cloudless days, between 09:00 and 15:00 h.

To compare a proportion of the biomass produced by each species over the season, we also collected the above-ground biomass of each phytometer. Research in these marshes determined that peak biomass for emergent marsh, meadow marsh, and *P. australis* vegetation communities occurred in mid-August 2016 and late July 2017 (Yuckin & Rooney 2019). In early

August 2016 and mid-July 2017, we collected the above-ground biomass of each phytometer by clipping them at the base of the stem, and dried and weighed the biomass. Due to clonal origin of the phytometers and the extent of below-ground biomass in the marsh it was impossible to determine what fraction of belowground biomass reflected growth during the study period.

2.2.3 Resource Measurements

To characterize niche overlap among species we collected data on environmental variables thought to define the niche from sites dominated by each of the phytometer species: *Typha* spp. (n = 15), invasive *P. australis* (n = 15), *C. canadensis* (n = 15), and *C. aquatilis* (n = 15) for a total of 60 sites. We selected areas where resident species were abundant and not experiencing direct interactions with *P. australis* to best approximate their realized niche within an extensively invaded marsh. Each of the sites were a minimum of 10 meters from one another and were situated between 1 and 1000 m from the location of the phytometers (Appendix 1A). At each site we collected a 10 cm deep soil core to measure soil nutrients which was then dried and homogenized into a powder. We also collected soil moisture using a WET sensor kit and HH2 moisture meter (Delta-T Devices, Burlington, ON), and the percent of PAR intercepted by the canopy by deploying the Li-Cor sensors described above to take simultaneous readings from the top of the canopy and the soil or water surface on cloudless days between 09:00 and 15:00 h.

2.2.4 Laboratory Analyses

We measured the $\delta^{13}C$ and $\delta^{15}N$ isotopic composition of phytometer leaves to determine photosynthetic performance and nitrogen sources, respectively. The plants in our study are C_3 photosynthesizers which have $\delta^{13}C$ values that range between -20 to -37‰ (Kohn 2010). The C_3

photosynthetic pathway discriminates against the heavier ^{13}C isotope during stomatal diffusion and carboxylation by Rubisco (Fry 2006, O'Leary 1988) - plants that discriminate less between C isotopes typically photosynthesize more efficiently and have a higher (less negative) $\delta^{13}C$ value (Farquhar et al. 1989, McAlpine et al. 2008). We also measured the carbon (% dry weight) and nitrogen (% dry weight) content in the same leaves to relate photosynthetic performance to nutrient content (e.g., Hirose and Werger 1994, Hirtreiter and Potts 2012). We selected a subset of 48 phytometers from the 2016 season: ten individuals of each of the resident species (5 'with competition,' and 5 'without competition') (10 x 3 = 30 samples), and six *P. australis* phytometers (3 'with competition,' 3 'without competition') for each of the neighbouring species (6 x 3 = 18 samples). Each phytometer was dried at 80^{o}C for 24 hours, then a random selection of leaf material was ground into a homogenous powder (1 mg). Every fifth sample was duplicated for precision quality control/quality assurance. The samples then underwent combustion conversion to gas through a 1108 Elemental Analyzer (Fisons Instruments) coupled to a Delta Plus XL (Thermo-Finnigan, Germany) continuous flow isotope ratio mass spectrometer. The $\delta^{13}C$ values were corrected to the primary reference scale of Vienna Pee Dee Belemnite, whereas the $\delta^{15}N$ values were corrected against the primary reference scale of Atmospheric Air. The %N and %C element content is a bulk measurement based on the sample weight against known certified elemental standards. Analyses were conducted by the Environmental Isotope Laboratory at the University of Waterloo. Three of the duplicate samples (two *C. canadensis*, one *P. australis*) were outside of the calibration range and were removed from the dataset, leaving seven duplicates for precision analysis. Analytical precision was measured using relative percent difference between duplicates (EPA 2014), and the average

precision was: 3.25% (± 3.12 st. error) for %C, 4.89% (± 3.83 st. error) for %N, 0.002% (± 0.113 st. error) for $\delta^{13}C$ and 8.88% (± 5.24 st. error) for $\delta^{15}N$.

Each soil sample collected from the unmanipulated areas was dried at 80^{o}C, ground into a homogenous mixture, then analyzed for soil pH, phosphorus (mg/Kg), carbon (% dry weight), total nitrogen (% dry weight), calcium (mg/Kg), potassium (mg/Kg), magnesium (mg/Kg), sodium (mg/Kg), copper (mg/Kg), iron (mg/Kg), zinc (mg/Kg dry), manganese (mg/Kg), and sulfur (µg/g). Plant available phosphorus was measured using sodium bicarbonate-extractable phosphorus following Reid (1998). Total nitrogen (TN) and carbon were measured using thermal conductivity detection (Reid 1998). The K, Mg, Ca, and Na samples were extracted using 1.0N Ammonium Acetate solution, following Simard (1993). Copper, iron, and zinc samples were extracted using a 0.005M DTPA solution and the filtrate was analyzed by ICP-OES following Liang and Karamanos (1993). Manganese was measured using 0.1 N phosphoric acid as the extracting solution, following Reid (1998). For sulphur, homogenized samples were closed-vessel microwave digested with nitric acid and hydrochloric acid, then the microwave digested sample was brought to volume with Nanopure water and quantification was performed using ICP-OES (AOAC 2011.14). Nitrogen and carbon analyses were done at the Biogeochemical Analytical Service Laboratory at the University of Alberta while the other nutrient analyses were conducted by the Agriculture and Food Laboratory at the University of Guelph.

2.2.5 Statistical Analyses

We determined that year did not influence plant biomass (general linear model: $F_{1,164}$ = 2.40, p = 0.123) or carbon assimilation rates at full sunlight (1500 µmol s^{-1} m^{-2}) (general linear model: $F_{1, 164}$ = 0.004, p = 0.948), so we pooled the data from both years for these variables. We

used two-way ANOVAs to compare carbon assimilation rates and water use efficiency at 1500 µmol s-1 m-2 among species and between the treatments. We ran four models in total with either carbon assimilation rate or water use efficiency as the response variable, with an interaction between phytometer species (for resident phytometers) or phytometer neighbours (for *P. australis* phytometers) and treatment (with or without competition). We used the same model design to assess differences in δ15N, δ13C, %N, and %C, conducting eight two-way ANOVAs in total. Duplicates in the carbon, nitrogen, and isotope data from phytometer leaves were averaged to one value for analyses. We used Type III sums of squares, unless an interaction was not significant, then we report Type II sums of squares. If a fixed factor was significant, without a significant interaction term, we used Tukey's HSD post-hoc test to assess for differences among levels of the factor. Analyses were performed using the car package (Fox and Weisberg 2019) and agricolae (de Mendiburu 2020) using R v. 4.0.3 (R Core Team 2020).

To evaluate the allocation of resources to above-ground biomass between treatments we compared the yield of phytometers growing with competition to those growing without competition. Using the phytometers that were paired by height at the beginning of the experiment, we calculated differences in above-ground yield using the relative competition index (RCI) approach (Grace 1995, Goldberg et al. 1999, Vilà and Weiner 2004):

$$RCI = (Y_{\text{without competition}} - Y_{\text{with competition}})/Y_{\text{without competition}}$$

Yield (Y) represents the above-ground biomass of each phytometer. As the weights are standardized, values greater than 0, with a maximum of 1, indicate that the above-ground biomass of the plant growing with competition was lower than its counterpart growing without competition. Values < 0 indicate the above-ground biomass of the plant growing with competition was higher than its counterpart growing without competition. This allows us to

compare the differences in above-ground yield among the species while accounting for variation in size among species.

All soil variables, except pH and light, were converted to ppm (i.e., mg/Kg) and log transformed to improve normality. To control for collinearity among environmental variables, we summarized the underlying correlation structure using principal components analysis (PCA). We created a matrix of all the soil nutrients, pH, soil moisture, and proportion of incident PAR reaching the ground and conducted the PCA, with a correlation matrix, using the rda function in vegan (Oksanen et al. 2020). The PCA scores were then multiplied by the proportion of variance explained by each axis, to give them appropriate weight, and were used as an indicator of ecological niche to quantify trophic niche region and overlap among the plant species using nicheROVER (Lysy et al. 2017). To estimate pairwise niche overlap, nicheROVER employs a Bayesian framework to calculate the probability that an individual from species A is found in the niche region (a 95% probability region in multivariate space) of species B (Swanson et al. 2015). All analyses were performed using R v. 4.0.3 (R Core Team 2020).

2.3 Results

2.3.1 Resident species carbon assimilation and water use efficiency

Carbon assimilation rates in resident species showed a marked effect of competition (Fig. 2.1). From PAR levels 200 µmol s^{-1} m^{-2} to 1500 µmol s^{-1} m^{-2} carbon assimilation rates were higher in resident species growing without competition compared to those growing with competition (Fig. 2.1A). While there was no interaction between species and treatment (two-way ANOVA $F_{2,91} = 0.759$, $p = 0.471$), all three resident species had higher carbon assimilation rates at 1500 µmol s^{-1} m^{-2} PAR when they were growing without competition (average 15.6 µmol

CO_2 s^{-1} m^{-2} (± 0.74 st. error)) than with competition (average 10.9 µmol CO_2 s^{-1} m^{-2} (± 0.73 st. error)) (two-way ANOVA $F_{1,91} = 33.52$, $p < 0.001$; Fig. 2.1C). There was also a significant difference in carbon assimilation rates among the resident species (two-way ANOVA $F_{2,91} = 38.720$, $p < 0.001$). *Typha* spp. (average 19.2 µmol CO_2 s^{-1} m^{-2} (± 1.07 st. error)) had carbon assimilation rates that were significantly higher than either *C. canadensis* (10.8 µmol CO_2 s^{-1} m^{-2} (± 0.54 st. error)) or *C. aquatilis* (11.8 µmol CO_2 s^{-1} m^{-2} (± 0.92)) (Fig. 2.1C).

Water use efficiency in resident species followed the same pattern as carbon assimilation rates. There was no significant interaction between species and treatment (two-way ANOVA $F_{2,91} = 1.737$, $p = 0.181$), however species (two-way ANOVA $F_{2,91} = 3.146$, $p = 0.0477$) and treatment (two-way ANOVA $F_{1,91} = 9.22$, $p = 0.003$; Fig. 2.1D) were both significant. Phytometers growing without competition had higher water use efficiency (2.32 mmol CO_2 mol^{-1} H_20 (± 0.07 st. error)) than those growing with competition (2.00 mmol CO_2 mol^{-1} H_20 (± 0.08 st. error)). As with carbon assimilation rates, *Typha* spp. had the highest water use efficiency (2.38 mmol CO_2 mol^{-1} H_20 (± 0.11 st. error)). *Calamagrostis canadensis* had the lowest water use efficiency (2.05 mmol CO_2 mol^{-1} H_20 (± 0.07 st. error)) and was significantly lower than *Typha* spp., while *C. aquatilis* was not significantly different than either species (2.12 mmol CO_2 mol^{-1} H_20 (± 0.12 st. error) (Fig. 2.1D).

2.3.2 Phragmites australis *carbon assimilation and water use efficiency*

There was no difference in carbon assimilation rates for *P. australis* growing with or without competition (Fig. 2.1B). There was no significant interaction between neighbouring species or treatment (two-way ANOVA $F_{2,63} = 0.615$, $p = 0.544$), and neither treatment (two-way

ANOVA $F_{1,63} = 2.80$, $p = 0.099$) or neighbouring species (two-way ANOVA $F_{2,63} = 1.459$, $p = 0.240$) were significant predictors of carbon assimilation rates in *P. australis* (Fig. 2.1E). The average carbon assimilation rate of *P. australis* at 1500 µmol s^{-1} m^{-2} (19.6 µmol CO_2 s^{-1} m^{-2} (± 0.51 st. error)) was similar to *Typha* spp. (19.2 µmol CO_2 s^{-1} m^{-2} (± 1.07 st. error)), and considerably higher than *C. aquatilis* or *C. canadensis.* Average water use efficiency between treatments did not vary among neighbouring species (two-way ANOVA $F_{2,63} = 0.064$, $p = 0.938$), nor among neighbouring species (two-way ANOVA $F_{2,63} = 2.026$, $p = 0.141$). Water use efficiency was significantly different between the treatment types (two-way ANOVA $F_{1,63} = 4.036$ $p = 0.0488$) and was higher when growing without competition from resident species. On average, the WUE of *P. australis* was considerably higher than the WUE of three resident species (average 3.0 mmol CO_2 mol^{-1} H_20 (± 0.12 st. error)) (Fig. 2.1F).

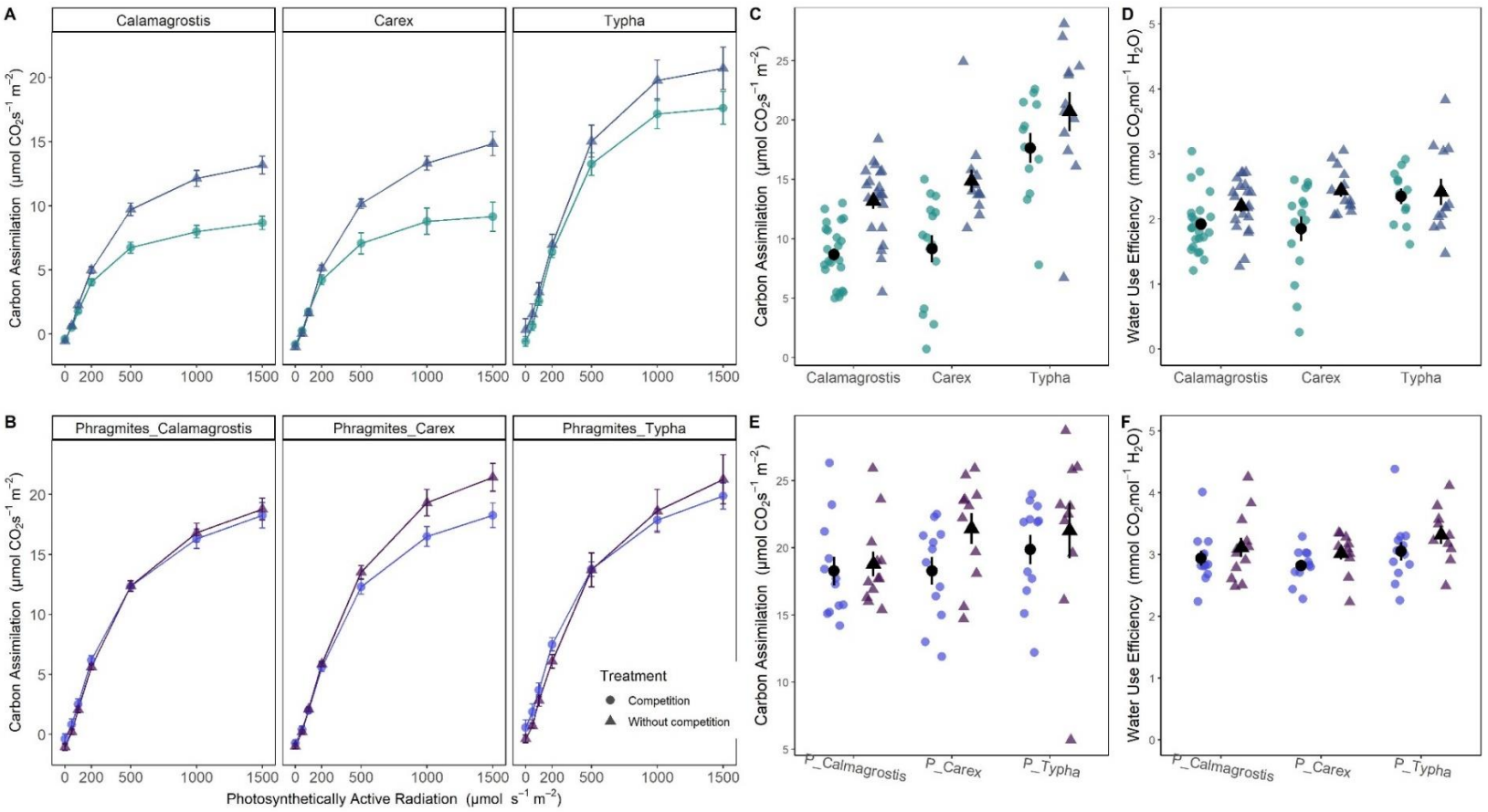

Figure 2.1 The carbon assimilation rates (µmol CO2 s^{-1} m^{-2}) along a photosynthesis-irradiance curve for resident plant species (A) and *P. australis* phytometers (B) with or without above-ground competition. Differences in carbon assimilation rates (µmol CO2 s^{-1} m^{-2}) and water use efficiency (mmol CO_2 mol^{-1} H_2O) at 1500 µmol s^{-1} m^{-2} are illustrated for resident species (C & D) and P. australis phytometers (E & F). Black points represent the mean and error bars in all panels are the standard error.

2.3.3 Relative Competition Index results

Unlike carbon assimilation, aboveground RCI values did not exhibit a clear effect of competition. The average aboveground RCI values for resident species ranged from positive, indicating competition, to negative, indicating facilitation (Fig. 2.2A). *Carex aquatilis* had the highest average aboveground RCI (0.156 (± 0.069 st. error)), while *C. calamagrostis* had the lowest (-0.651 (± 0.552 st. error)). However, all of the *Phragmites australis* aboveground RCI values were negative, suggesting mild facilitation when growing with resident species (Fig. 2.2B). The amount of PAR reaching resident species was, on average, higher when growing without competition from *P. australis* (Table 2.1; Appendix 1F). When growing without competition the percent of incident PAR reaching *C. canadensis* was 26.6% higher, the percent reaching *C. aquatilis* was 8.6% higher, and the percent reaching *Typha* spp. was 14.8% higher. *Phragmites australis* also received more PAR on average when growing without competition (Table 2.1). Overall, *P. australis* (21.0 g (± 1.45 st. error), n = 66) and *Typha* spp. (37.5 g (± 2.13 st. error), n = 24) generated considerably more aboveground biomass than *C. canadensis* (1.87 g (± 0.29 st. error), n = 43) and *C. aquatilis* (2.09 g (± 0.19 st. error), n = 33) (Appendix 1F). Over the field season, *Typha* spp. and *P. australis* also gained height much faster than *C. canadensis* and *C. aquatilis* (Appendix 1G). On average, phytometers growing without competition did not grow taller than those growing with competition. In fact, resident meadow species (*C. canadensis* and *C. aquatilis)* were shorter when growing without competition, though this appeared to differ between 2016 and 2017. At the end of the 2016 growing season, and throughout the 2017 season, *Typha* spp. growing without competition was also shorter than *Typha* spp. growing with competition (Appendix 1G).

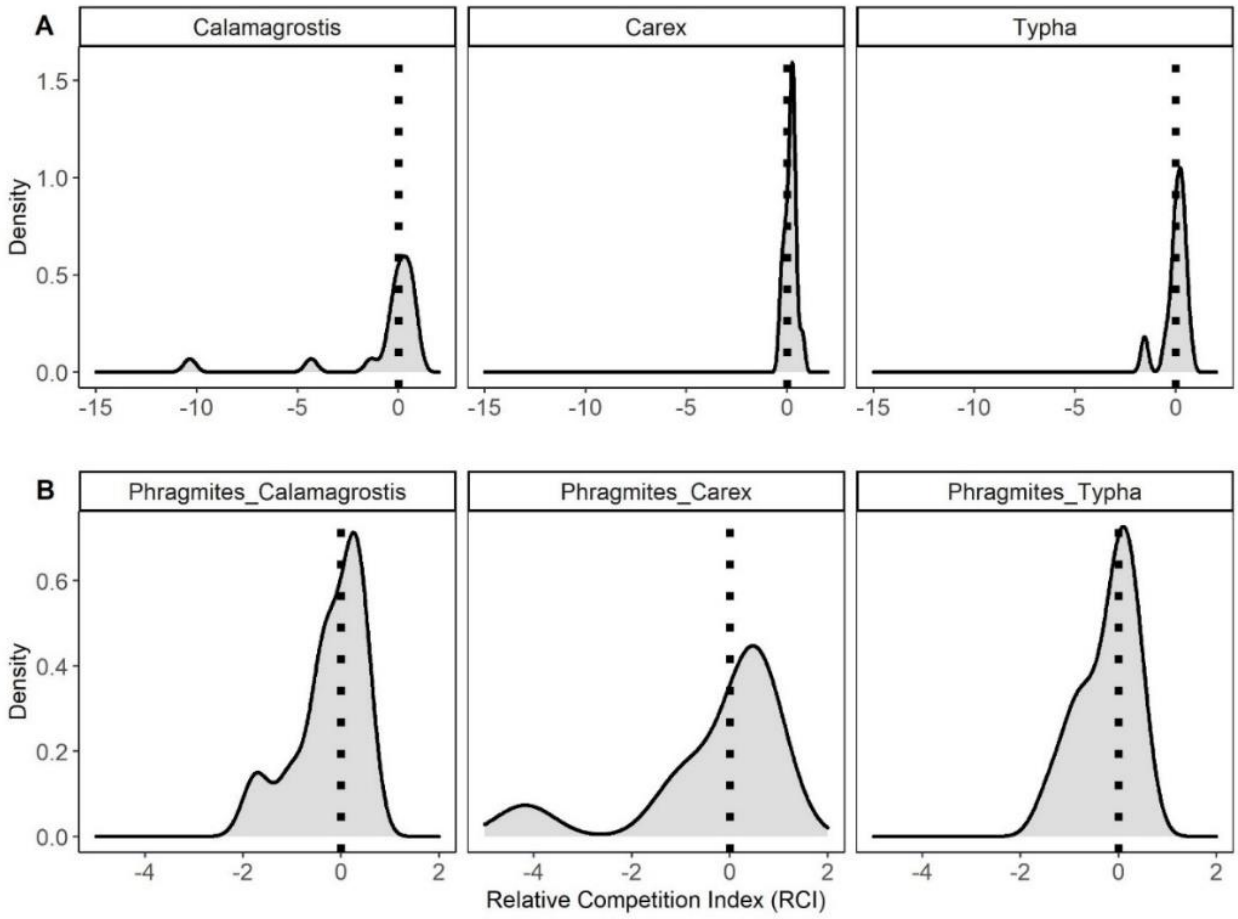

Figure 2.2. The relative competition index of resident species (A) and *P. australis* growing with resident neighbours (B). Values above 0 indicate an effect of competition, with a maximum value of 1, while values below 0 indicate facilitation. Data includes phytometers from 2016 and 2017, collected over the course of one growing season (May to July) in Long Point, ON.

2.3.4 Resident species foliar nutrient content and isotopes

Overall, resident species in both treatments had lower nitrogen to carbon ratios than invasive *P. australis* phytometers growing without competition (Fig. 2.3A). Differences in foliar carbon content among species did not vary between treatments ($F_{2,23} = 2.422$, $p = 0.493$), nor was treatment significant ($F_{2,23} = 0.229$, $p = 0.714$). However, there was a significant difference in carbon content among the resident plant species ($F_{2,23} = 27.31$, $p < 0.01$) (Fig. 2.3C; Table 2.2).

The carbon content in *Typha* spp. (average 48.4% dry weight (± 0.42 st. error)) was significantly higher than *C. aquatilis* (average 46.1% dry weight (± 0.27 st. error)), while *C. canadensis* was intermediate (average 47.5% dry weight (± 0.50 st. error)) (Table 2.1). Nitrogen content in the leaves of resident species exhibited a significant interaction between species and treatment ($F_{2,23}$ = 3.770, p = 0.038; Figure 2.3D). Nitrogen content in *C. aquatilis* and *C. canadensis* increased when phytometers grew without competition, while *Typha* spp. nitrogen content decreased when growing without competition (Fig. 2.3D).

The mean $\delta^{13}C$ value among species did not differ between treatments ($F_{2,23}$ = 0.044, p = 0.956), but $\delta^{13}C$ values were significantly different among species ($F_{2,23}$ = 17.922, p < 0.001) and between treatments ($F_{1,23}$ = 10.342, p = 0.004). The average $\delta^{13}C$ value in *Typha* spp. leaves (-29.6 ‰ (± 0.254 st. error)) was significantly lower than *C. aquatilis* (-28.3 ‰ (± 0.268 st. error)) and *C. canadensis* (-27.6 ‰ (± 0.341 st. error)). The average $\delta^{13}C$ value for phytometers growing with competition was lower (-29.0 ‰ (± 0.316 st. error)) than those without competition (-28.0 ‰ (± 0.294 st. error)). In contrast, the average $\delta^{15}N$ values among species did not differ between treatments ($F_{2,23}$ = 0.339, p = 0.716), and did not differ between treatments ($F_{1,23}$ = 2.424, p = 0.133) or among species ($F_{2,23}$ = 2.017, p = 0.156) (Fig. 2.3E). The average $\delta^{15}N$ for *Typha* spp. was 1.93‰ (± 0.254 st. error), for *C. canadensis* was 2.40‰ (± 0.508 st. error), and 2.91‰ (± 0.270 st. error) for *C. aquatilis.*

2.3.5 Phragmites australis *foliar nutrient content and isotopes*

For *P. australis* phytometers, carbon content among neighbouring species did not differ between treatments, nor between treatments or among neighbouring species ($F_{2,12} \leq 3.576$, $p \geq 0.083$; Fig. 2.3C, Table 2.2). The average carbon content in *P. australis* (46.94% by weight (± 0.22 st. error)) was lower than *Typha* spp., but similar to *C. aquatilis.* The nitrogen content in *P.*

australis phytometers differed significantly between treatments ($F_{1,12} = 18.783$, $p = 0.001$), but the interaction term ($F_{2,12} \leq 0.010$, $p = 0.990$) and neighbours were not significant predictors ($F_{2,12} = 0.462$, $p = 0.641$) (Fig. 2.3D). The nitrogen content in phytometers growing without competition was significantly higher (2.47% by weight (± 0.12 standard error)) than those growing with competition (1.75% by weight (± 0.09 standard error)). The interaction term and fixed factors were not significant predictors of $\delta^{13}C$ values ($F_{2,12} \leq 1.388$, $p \geq 0.262$; Table n) or $\delta^{15}N$ values ($F_{2,12} \leq 2.367$, $p \geq 0.136$; Table 2.2) in *P. australis* phytometers. The average $\delta^{13}C$ value was -26.58 ‰ (± 0.18 st. error) and average $\delta^{15}N$ value was 4.04 ‰ (± 0.20 st. error) (Fig. 2.3E). The average $\delta^{15}N$ values were higher in *P. australis* than any of the resident species (Fig. 2.3F, Table 2.1).

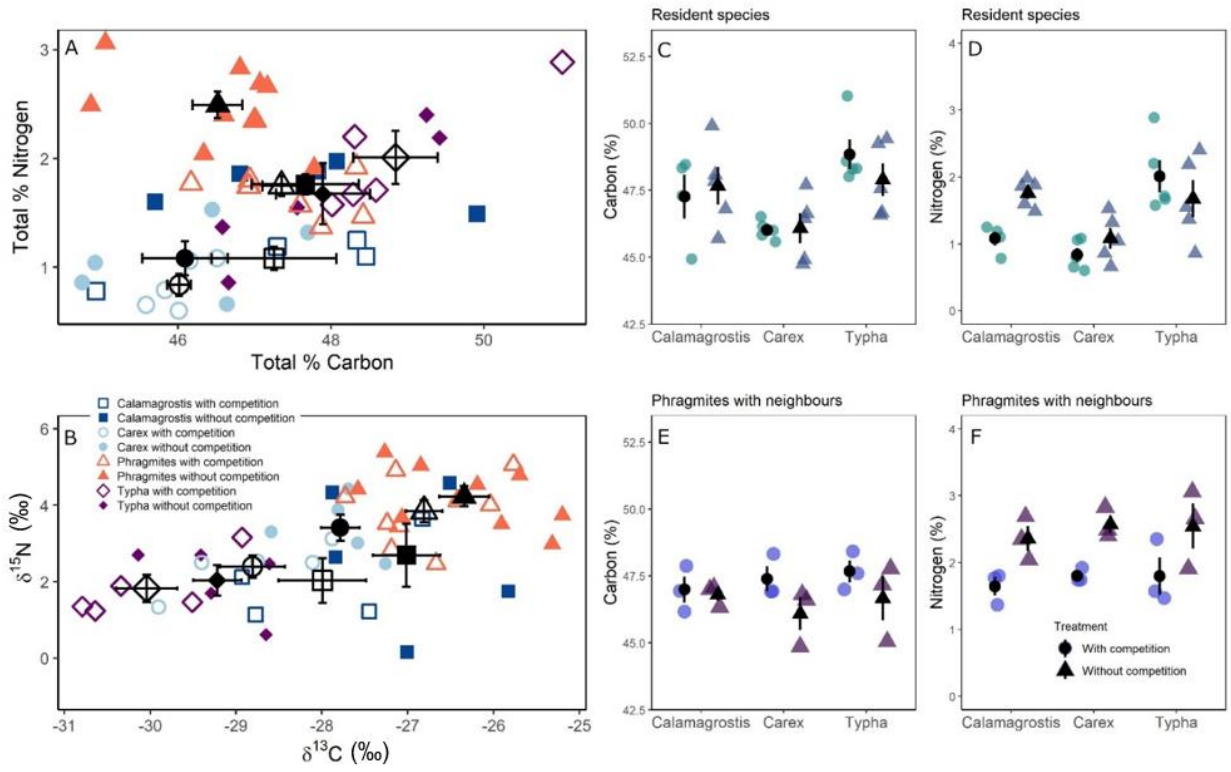

Figure 2.3. Scatter plots illustrating the carbon (%) and nitrogen (%) content in phytometer leaves (A) and the $\delta^{15}N$ and $\delta^{13}C$ ratios for each phytometer (B). Large points represent the mean and error bars represent standard error for each phytometer and treatment type. The jitter plots show the carbon and nitrogen content in resident phytometers (C & D) and *P. australis* phytometers (E & F) growing with or without above-ground competition, with means and standard errors symbolized.

Table 2.1 Averages and standard errors for response variables for each phytometer and treatment: average aboveground relative competition intensity (RCI) where values from 0 – 1 indicates competition, and values below 0 indicate facilitation; percent of photosynthetically available radiation (µmol s^{-1} m^{-2}) (PAR) reaching each phytometer; the amount of above-ground biomass (g) generated by each phytometer; carbon assimilation rate (µmol CO_2 s^{-1} m^{-2}) (A) at 1500 µmol s^{-1} m^{-2} PAR; water use efficiency (CO_2 mmol s^{-1} m^{-2} H_20) (WUE) at 1500 µmol s^{-1} m^{-2} PAR; percent carbon (%C), nitrogen (%N), $\delta^{13}C$ and $\delta^{15}N$ values from leaves.

		Calamagrostis candensis	*Carex aquatilis*	*Typha* spp.	*Phragmites australis* and *C. canadensis*	*Phragmites australis* and *C. aquatilis*	*Phragmites australis* and *Typha* spp.
PAR %	Without competition	67.0 (± 6.51)	50.0 (± 5.47)	90.0 (± 2.14)	91.6 (± 2.35)	90.6 (± 2.00)	94.4 (± 1.86)
	Competition	40.4 (± 6.74)	41.4 (± 6.78)	75.2 (± 7.03)	77.3 (± 8.37)	82.6 (± 7.54)	93.7 (± 1.63)
Biomass (g)	Without competition	2.24 (± 0.541)	2.2 (± 0.223)	38.8 (± 2.38)	25.7 (± 4.59)	16.7 (± 2.19)	21.1 (± 2.89)
	Competition	1.47 (± 0.161)	2.0 (± 0.305)	36.3 (± 3.60)	27.8 (± 4.96)	15.2 (± 3.19)	19.6 (± 2.32)
A	Without competition	13.2 (± 0.69)	14.9 (± 0.94)	20.7 (± 1.65)	18.8 (± 0.91)	21.4 (± 1.16)	19.9 (± 1.09)
	Competition	8.7 (± 0.51)	9.2 (± 1.14)	17.6 (± 1.28)	18.3 (± 1.06)	18.3 (± 1.03)	21.3 (± 2.05)
WUE	Without competition	2.2 (± 0.09)	2.44 (± 0.09)	2.42 (± 0.20)	3.11 (± 0.16)	3.02 (± 0.10)	3.32 (± 0.14)
	Competition	1.9 (± 0.09)	1.85 (± 0.19)	2.35 (± 0.12)	2.94 (± 0.12)	2.82 (± 0.08)	3.05 (± 0.15)
%C	Without competition	47.3 (0.82)	46.0 (0.16)	48.9 (0.55)	46.8 (± 0.23)	46.1 (± 0.62)	46.7 (± 0.83)
	Competition	47.7 (0.70)	46.1 (0.56)	47.9 (0.61)	47.0 (± 0.49)	47.4 (± 0.47)	47.7 (± 0.41)
%N	Without competition	1.1 (0.10)	0.8 (0.10)	2.0 (0.24)	2.4 (± 0.19)	2.6 (± 0.13)	2.5 (± 0.34)
	Competition	1.8 (0.09)	1.1 (0.16)	1.7 (0.28)	1.7 (± 0.14)	1.8 (± 0.06)	1.8 (± 0.28)
$\delta^{13}C$	Without competition	-27.9 (0.51)	-28.8 (0.38)	-30.0 (0.36)	-26.5 (± 0.66)	-26.4 (± 0.60)	-26.1 (± 0.44)
	Competition	-27.0 (0.39)	-27.8 (0.22)	-29.2 (0.28)	-27.0 (± 0.50)	-27.0 (± 0.16)	-26.4 (± 0.40)

Table 2.2 ANOVA results for carbon assimilation (µmol CO2 s^{-1} m^{-2}) (A) and water use efficiency (CO_2 mmol s^{-1} m^{-2} H_20) (WUE) at 1500 µmol s^{-1} m^{-2} PAR, percent carbon (%C), nitrogen (%N), $\delta^{13}C$ and $\delta^{15}N$ values from phytometer leaves. Resident species phytometers (*C. aquatilis, C. canadensis, Typha* spp.) or *P. australis* phytometer neighbours (*C. aquatilis, C. canadensis, Typha* spp.) and treatment (with competition or without competition) as fixed factors.

	Resident species phytometers										*Phragmites australis* phytometers									
	Species			Treatment			Spp x Treatment			Residual	Neighbours			Treatment			Neighbour x Treatment			Residual
	df	F	p	df	F	p	df	F	p		df	F	p	df	F	p	df	F	p	
A	2	38.72	<0.001	1	33.52	<0.001	2	0.76	0.471	91	2	1.45	0.240	1	2.80	0.099	2	0.62	0.544	63
WUE	2	3.15	0.048	1	9.22	0.003	2	1.74	0.182	91	2	2.03	0.140	1	4.04	0.048	2	0.06	0.938	63
%C	2	8.23	0.002	1	0.138	0.714	2	0.730	0.493	23	2	0.32	0.730	1	3.58	0.083	2	0.57	0.581	12
%N	2	11.54	<0.001	1	6.32	0.019	2	3.77	0.038	23	2	0.46	0.641	1	18.78	<0.001	2	0.01	0.991	12
$\delta^{13}C$	2	17.92	<0.001	1	10.34	0.004	2	0.05	0.956	23	2	0.56	0.584	1	1.39	0.262	2	0.04	0.963	12

2.3.6 Niche space and niche overlap

The first two axes of the PCA accounted for 75% of the variance in the data (Axis 1: 0.667, Axis 2: 0.080), and were selected based on a scree plot assessing inertia (Appendix 1H-J). PCA axis 1 illustrates a clear gradient among the four species, with *C. canadensis* on one end and *Typha* spp. on the opposite (Appendix 1H). *Calamagrostis canadensis* had the lowest average soil moisture (73.6 % (±1.53 st. error, coefficient of variation 8.1%), while *C. aquatilis* (79.2% (± 0.93 st. error, coefficient of variation 4.5%)) and *Typha* spp. (82.0% (± 1.29 st. error, coefficient of variation 6.1%)) had higher soil moisture. *Phragmites australis* sites had an average soil moisture of 78.2% (± 3.84 st. error) with the largest range (coefficient of variation 19.0%) that overlapped the other species. Soils where *Typha* spp. dominated also had the highest concentrations of phosphorous (35.6 mg/kg (± 5.23 st. error)), potassium (128.6 mg/kg (± 0.11 st. error)), and nitrogen (11,066.7 mg/kg (± 1277.26 st. error)), while soils where *C. canadensis* dominated had the lowest concentration of these three nutrients (P: 9.2 mg/Kg (± 0.75 st. error), K: 34.15 mg/kg (± 0.3.05 st. error), N: 1780 mg/kg (± 154.06 st. error)). PCA axis 2 seems to illustrate a gradient between incident light reaching the ground. The average incident PAR reaching the ground was 16.07% (± 5.81 st. error) in stands of *C. aquatilis,* 8.09% (± 2.09 st. error) in stands of *C. canadensis,* 6.21% (± 1.55 st. error) in stands of *Typha* spp., and 2.76% (± 0.83 st. error) in stands of *P. australis.*

Using the scores from the PCA, multiplied by the variance explained by each axis, we determined the probabilistic (95%) niche region and niche overlap for each species. *Phragmites australis* (0.360 (± 0.10 st. error)) had the largest niche region of the four species, followed by *Typha* spp. (0.304 (± 0.08 st. error)), *C. aquatilis* (0.186 (± 0.05 st. error)), then *C. canadensis* (0.077 (± 0.02 st. error)) (Fig. 2.4A). The probability of *P. australis* overlapping with the niche

space of *C. aquatilis* (median 64.3 (42 - 87 credible interval)) and *Typha* spp. (median 53.5 (22 - 84 credible interval)) was higher than the probability of overlap with *C. canadensis* (median 8.3 (2 - 22 credible interval)). However, the probability of overlap of the three resident species onto the niche of *P. australis* was higher: a median of 83.6 (25 - 100 CI) for *C. canadensis*, a median of 94.9 (73 - 100 CI) for *C. aquatilis*, and a median of 53.4 (30 - 93 CI) with *Typha* spp. (Fig. 2.4B; Table 2.3).

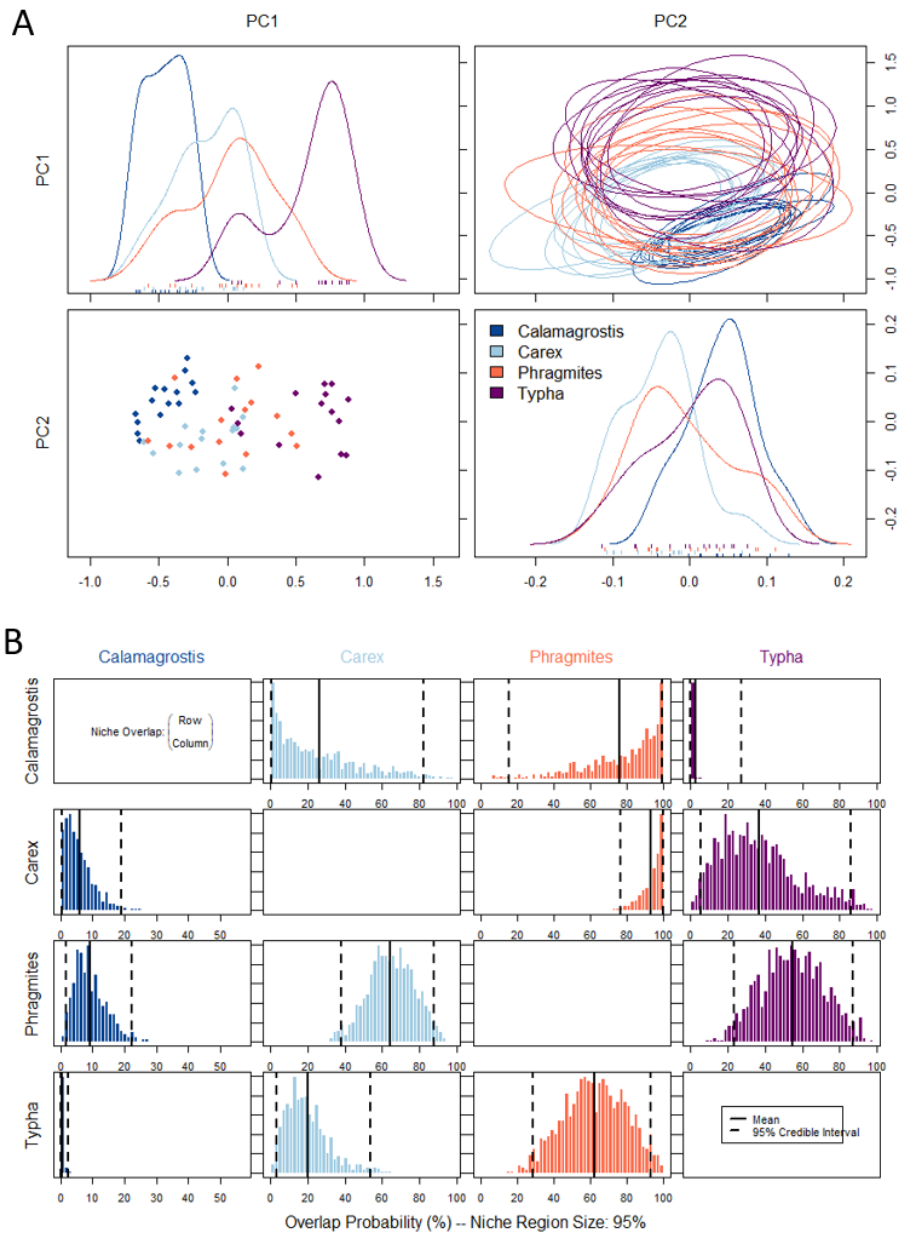

Figure 2.4. Projections of the 95% niche regions for each plant species based on weighted PCA axis scores (A); quadrats include one-dimensional density plots and 10 randomly drawn ellipticals per species which represent the two-dimensional niche region (A). Posterior distribution of the probabilistic niche overlap metric (%) for specific 95% niche region of all four plant species. Plots show the overlap probability of species A (row) onto the niche of species B (column). The posterior means and 95% credible intervals are displayed in black.

Table 2.3. Estimated niche region size, with standard error, and median overlap of 95% niche region with 95% credible intervals for invasive *P. australis* and resident *C. canadensis*, *C. aquatilis*, and *Typha* spp. based on PCA scores. Values indicate the overlap probability of species A onto the niche of species B.

Species A	95% Niche Region	Species B	Median & 95% CI
C. canadensis	0.077 (± 0.021)	*C. aquatilis*	20.2 (0 – 83)
		Typha spp.	0.0 (0 – 34)
		P. australis	83.6 (25 – 100)
C. aquatilis	0.186 (± 0.048)	*C. canadensis*	4.7 (0 – 18)
		Typha spp.	31.9 (0 – 34)
		P. australis	94.9 (73 – 100)
Typha spp.	0.304 (± 0.083)	*C. canadensis*	0.1 (0 – 2)
		C. aquatilis	16.5 (3 – 51)
		P. australis	53.4 (30 – 93)
P. australis	0.360 (± 0.098)	*C. canadensis*	8.3 (2 – 22)
		C. aquatilis	64.3 (42 – 87)
		Typha spp.	53.5 (22 – 84)

2.4 Discussion

Detrimental invasive species should exhibit high niche overlap and large fitness differences with resident species (MacDougal et al. 2009). Using a common invasive species, we quantified differences in photosynthetic performance, competition for photosynthetically active radiation, and niche overlap between a globally successful invasive species, *P. australis*, and resident wetland species. Invasive *P. australis* intercepted more photosynthetically active radiation than resident plants and had higher carbon assimilation rates and photosynthetic efficiency than resident species. Direct competition with *P. australis* significantly reduced the photosynthetic performance of resident species, while competition with resident species did not

negatively impact *P. australis*. *Phragmites australis* also has the largest niche region and was most likely to overlap onto the niche of *C. aquatilis* and *Typha*. Resident species had a higher probability of overlapping onto the niche space of *P. australis*, particularly the meadow marsh species *C. canadensis* and *C. aquatilis*. This finding agrees with historical records of the region that identified meadow marsh and grass/sedge hummock were frequently displaced by *P. australis* invasion (Wilcox et al. 2003). These results suggest *C. canadensis* and *C. aquatilis* could persist in invaded areas if they were not currently being excluded. Our results confirm that *P. australis* has high niche overlap with resident plant species, is not negatively affected by competition for above-ground resources with resident species and utilizes this limited resource more effectively than resident species. This study represents an advancement in testing the effects of fitness and niche differences in the success of a perennial, rhizomatous invasive plant.

The carbon assimilation rate and water use efficiency of all three resident species were lower than *P. australis* and showed a decrease when growing with above-ground competition. Meadow marsh species *C. canadensis* and *C. aquatilis* assimilated considerably less carbon and were less efficient at generating biomass per unit water transpired (Farquhar et al. 1989) than *P. australis*, and competition decreased carbon assimilation and water use efficiency further. Over the growing season *C. canadensis* and *C. aquatilis* produced less above-ground biomass and did not grow as tall or as fast as *P. australis*, a commonality among many species in *P. australis* invaded wetlands. In both freshwater and brackish marshes in North America, *P. australis* consistently produces more above-ground biomass than other wetland plants in the same system (Meyerson et al. 2000). *Typha*, however, also grows quickly, produces extensive above-ground biomass (Bansal et al. 2019), and has carbon assimilation rates similar to *P. australis.* However, *Typha* exhibited a decrease in carbon assimilation due to above-ground competition with *P.*

australis, while *P. australis* competing with *Typha* did not. Our measurement of percent incident light in abundant stands of each species demonstrated that *P. australis* intercepts more photosynthetically active radiation than any of the resident species, including *Typha*. Similar work in freshwater marshes found the same pattern in monocultures and mixed stands of *Typha* and *P. australis* – approximately 30% of full sunlight reached the litter layer in *Typha* stands compared to 10% in *P. australis* stands – which the authors attribute to the horizontal orientation of *P. australis* leaves (Hirtreiter and Potts 2012). While these values are higher than what we measured, they also observed the pattern of *P. australis* intercepting more sunlight than *Typha.*

While *P. australis* produces dense stands that reduce light availability for other species, it is also subject to self-shading. Once stands have reached sufficient density, smaller and thinner shoots die prematurely from within-stand competition for light (van Der Toorn and Mook 1982, Hara et al. 1993). We are unable to assess if intraspecific competition for PAR is greater than interspecific competition due to the limits of our experimental design, but we can assess each species photosynthetic activity and whole plant function over the field season using $\delta^{13}C$ values (Dawson et al. 2002). *Phragmites australis* had the highest (least negative) $\delta^{13}C$ values (-26.58‰ ± 0.18 st. error), indicating enrichment with the heavier carbon atoms and more efficient photosynthesis over the growing season (Farquhar et al. 1989). Resident species had lower $\delta^{13}C$ values, and competition with *P. australis* resulted in lower average $\delta^{13}C$ values for all resident species which coincides with their lower photosynthetic water use efficiency (Fry 1992). While water availability is not a limiting factor for wetland plants, balancing the trade-off between carbon gain and water loss to transpiration is under the biological control of plants via stomatal conductance. When growing with competition, resident plants were less efficient at generating biomass per unit water transpired (Farquhar et al. 1989). This reduced efficiency may further

negatively affect plants growing in competition as they allocate more resources to above-ground growth.

Many of the resident species growing with competition produced more above-ground biomass or were taller than their counterparts growing without competition. Measuring above-ground biomass has been used to assess effects of competition in the field (e.g., Güsewell and Edwards 1999), however this approach appears to fail to account for other effects of competition. Plants can respond to low light availability by allocating more resources to above-ground tissue, such as height and leaf area (Weiner 2004; Craine and Dybzinski 2013) but this trade-off has consequences. Allocating more resources to above-ground tissue can result in reduced net carbon gain per individual, as observed in our measurements of carbon assimilation, and it can impede the acquisition of water and soil nutrients, resulting in a diminished overall competitive ability (Aschehoug et al. 2016). The low nitrogen content in the leaves of C. canadensis and C. aquatilis growing with competition appear to support this relationship between competition, re-allocation of resources, and nutrient uptake. The low nitrogen content in the leaves of *C. canadensis* and *C. aquatilis* growing with competition appear to support this observation. Uddin et al. (2018) found that *P. australis*-invaded sites had higher concentrations of availble soil nutrients, including nitrogen and phosphorus, but *P. austrails* produced extensive belowground biomass and usurped nutrients from neighbouring species. This competition for soil resources, coupled with the need to grow taller, results in particularly challenging conditions for meadow marsh species. In species-rich fens in Switzerland, shading from *P. australis* did not appear to affect the performance of native species (Güsewell and Edwards 1999). However, plant performance was evaluated by clipping above-ground biomass (Güsewell and Edwards 1999) and based on our results this work may have missed that plants were assimilating less carbon when growing with

P. australis. Another limitation to our clip plot approach is that we could not ensure conditions for phytometers were identical nor control each variable that phytometers were exposed to, as can be done in a more controlled environment e.g., a greenhouse. The patterns we observed in carbon assimilation and compensatory growth indicate that collecting above-ground biomass in the field does not tell the full story of plant interactions, especially for rhizomatous plants.

In addition to more efficient photosynthesis, *P. australis* had a higher nitrogen to carbon ratio when growing without competition than the other resident species. High foliar nitrogen is commonly noted in *P. australis* studies, suggesting *P. australis* is more efficient at nitrogen uptake than other wetland species (Farnsworth and Meyerson 2003). In freshwater and brackish marshes *P. australis* had higher foliar nitrogen (2 - 4 % N) than *Typha* (1 - 2%N) (Meyerson 2000). This pattern was observed in our study as well, however competition did reduce *P. australis* foliar nitrogen from an average of 2.49 % N without competition to 1.74 % N with competition. In contrast, *Typha* foliar nitrogen increased when growing with competition from *P. australis* (average 2.01 % N) and decreased when growing without competition (1.67 % N). The nitrogen content in the leaves of *C. aquatilis* and *C. canadensis* were lower than *P. australis* and decreased further when growing with competition. Nitrogen is used in the creation of chloroplasts (Evans 1989) and plants can maximize their photosynthetic efficiency by allocating more nitrogen to sunny leaves high in the canopy (Hirose and Werger 1987). Therefore, one possible explanation is that *C. canadensis, C. aquatilis,* and *P. australis* allocated more nitrogen to all leaves when access to PAR was higher, while *Typha* may have been more strategic and re-allocated nitrogen to only sunny leaves. While we sampled a random subset of leaves, Hirtreiter and Potts (2012) found that *Typha* allocated nitrogen to leaves that received full sunlight while *P. australis* was not strategic with nitrogen and had high leaf nitrogen content, and therefore

consistent intermediate values of carbon assimilation, down the canopy (Hirtreiter and Potts 2012). Another possible explanation is that *C. aquatilis, C. canadensis* and *P. australis* allocate more resources to height or non-leaf tissue when growing with competition and reduce the amount of nitrogen allocated to photosynthetic processes. In the case of *C. aquatilis* and *C. canadensis,* which are likely more N-limited than *P. australis,* this re-allocation of nitrogen away from photosynthetic processes reduced their carbon assimilation capacity (e.g., Aschehoug et al. 2016).

While it is difficult to discern the pathways that lead to $\delta^{15}N$ signatures in the field (Craine et al. 2015), *P. australis* had notably higher $\delta^{15}N$ values than resident species. While we were unable to directly assess plant available nitrogen, previous work in our study area has identified high organic matter content in the soil (Polowyk 2020). The isotopic composition of soil organic matter is enriched in $\delta^{15}N$ as decay and microbial changes occur (Craine et al. 2015). Therefore, it is possible that the high $\delta^{15}N$ values of *P. australis* suggests access to these nutrient pools or a better ability to take up nitrogen in the system. This is supported by recent research that determined there were higher concentrations of plant-available nutrients in the soil in *P. australis*-invaded areas (Uddin et al.-2018). However, it is unlikely that *P. australis* is accessing a different pool of nutrients than resident species via a physical characteristic such as rooting depth, as evidence from Long Point determined *P. australis* does not root more deeply than resident species (Lei et al. 2019). In contrast with *P. australis*, *Typha* had the lowest $\delta^{15}N$ values and both meadow species had substantially lower $\delta^{15}N$ values than *P. australis.* These values could indicate nitrogen limitation for resident species and reliance on denitrification (Craine et al. 2015), though *Typha* is documented to rapidly takes up nutrients in nutrient-rich

environments (Cicek et al. 2006) and establishes in wetlands with high nitrogen and phosphorus concentrations (e.g., Bansal et al. 2019).

Competition with *P. australis* has a detrimental effect on resident plants, and the niches of resident species are very likely to overlap with the niche of *P. australis*. The niches of the resident species were aligned along a gradient, with *C. canadensis* in an area of lowest soil moisture and nutrient concentration and *Typha* in areas of high soil moisture and nutrient concentrations. Wetland vegetation typically aligns itself along a topographical or moisture gradient, from inundated flooded areas to shallow standing water (Keddy 2010). Of the three resident species, *Typha* had the largest niche, which was slightly smaller than *P. australis,* and *C. canadensis* had the smallest niche. *Typha* x *glauca* and *Typha angustifolia,* a parent species of *Typha* x *glauca,* have a wide range globally, proliferate in high nutrient environments, and generate monocultures that alter wetland communities (Bansal et al. 2019). The large niche of *Typha* corroborates the well-documented niche breadth of this species. *Typha* x *glauca* and *Typha angustifolia* are also obligate wetland plants that are well adapted to deeper water and require a degree of consistent flooding (Bansal et al. 2019). This explains the low probability of niche overlap between *Typha* and meadow species *C. aquatilis* and *C. canadensis,* which preferentially establish in areas with shallower seasonal inundation (Darris 2006, Tilley et al. 2011). The median probability of niche overlap between *Typha* and *P. australis* were nearly identical for each species. However, when monospecific stands of *Typha* and *P. australis* grow together, *P. australis* is not slowed by competition and usually displaces *Typha* (Paradis et al. 2014). A component of this successful displacement is likely the negative effect that competition with *P. australis* has on *Typha* carbon assimilation. While *P. australis* and *Typha* have many similarities and are both considered invasive in North American wetlands (Galatowitsch et al.

1999), they do respond differently to environmental conditions. Water depth variability can keep *Typha* populations low (Boers and Zedler 2008), while managed water levels that maintain a consistent degree of flooding encourage growth (Wilcox and Nichols 2008). In contrast, *P. australis* populations have been documented to increase with variable water depths (e.g., Wilcox et al. 2003). This is observable around the Great Lakes, where the unmanaged water levels of Lake Erie support higher *P. australis* populations in coastal wetlands, while the managed water levels of Lake Ontario make *Typha* a more problematic wetland invader (Chin et al. 2014).

The median probability of *P. australis* overlapping with the niche space of *C. aquatilis* and *Typha* spp. was higher than the probability of overlap with *C. canadensis.* These results agree with historical data that identified *Typha* spp. (33.8%), meadow marsh (31%) and grass/sedge hummock (10%) as the vegetation communities replaced by *P. australis* in Long Point (Wilcox et al. 2003). The median probability of *P. australis* overlapping with the niche space of *C. canadensis* was very low, possibly because remnant patches now occur only in areas less likely to be invaded by *P. australis.* In contrast, the median probability of overlap onto the niche of *P. australis* was high for *C. canadensis* and *C. aquatilis* indicating they could likely persist in invaded habitat, if they were not currently being excluded. The results of our experiment demonstrate that competition for light in a *P. australis* stand would be difficult for meadow species, and their reduced carbon assimilation efficiency would make it challenging for them to persist in dense stands. While *P. australis* had the largest niche space of the four species we measured, our measurements were taken from an extensively invaded marsh. It is therefore likely that, pre-invasion, the size and overlap of niches among these species would be different.

Understanding the specific outcomes of plant interactions can inform the patterns we observe in the field. However, the results of species interactions are often attributed to competition despite numerous other mechanisms that can confer an advantage to an introduced species (e.g., Richardson and Pyšek 2006). While these were outside the scope of our study, our work does add to the growing body of literature about *P. australis* (Meyerson et al. 2016) that can assist us in better understanding how introduced species maintain their prominence in the community once established. We expect that throughout its range *P. australis* has high niche overlap with resident species, and likely has additional fitness differences (e.g., allelopathy, faster nutrient uptake etc.) that contribute to its dominance over the species that it is replacing in wetlands. For wetland managers who may be interested in active revegetation after the removal of *P. australis*, the ability of *P. australis* to shade out and assimilate carbon more efficiently than many native species should be considered. Selecting native species with traits such as fast growth early in the season and the ability to rapidly capture carbon may allow them to prevent re-establishment or persist alongside *P. australis.* Native meadow marsh species, such as *C. canadensis* and *C. aquatilis*, are unlikely to prevent *P. australis* re-establishment. We recommend future work integrates population level changes over a longer time period than one growing season within invaded marshes, and explicitly measures the differences between interspecific competition and intraspecific competition. This will provide a better understanding of the processes that allow *P. australis* to remain abundant in an invaded marsh and give additional insight into which native North American species, if any, can stably coexist with *P. australis.*

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3 Effective suppression of established invasive *Phragmites australis* leads to secondary invasion in a coastal marsh.

3.1 Introduction

Globally, invasive species alter ecosystems in direct and indirect ways (Pyšek et al. 2020). The addition of one species can modify community structure and ecosystem functions (Simberloff et al. 2013), and the majority of studied invasive plants negatively impact other plants at the species and community level (Pyšek et al. 2012). Theoretically, the removal of an invasive plant should catalyze the recovery of native species and ecosystem processes. Yet, well-established invasive species can become entrenched in the ecosystem, altering their environment (D'Antonio and Meyerson 2002), such that their removal can result in unanticipated ecological changes.

In practice, although management often succeeds in suppressing the targeted invasive plant, secondary invaders are commonly the principal beneficiaries and native species recovery is limited (Pearson et al. 2016). The removal of invasive plants can thus trigger undesirable outcomes that are difficult to anticipate (e.g., González et al. 2017). Unfortunately, as many have already noted (e.g., Blossey 1999; Kettenring and Adams 2011; Hazelton et al. 2014; Zimmerman et al. 2018), invasive species treatment actions are rarely followed by adequate monitoring to provide accountability regarding invasive species management and native vegetation restoration outcomes. For these practices to be considered successful, they should achieve the recovery goals of land managers regarding native vegetation and not only the suppression of the invasive species that prompted the management action.

Phragmites australis ssp. *australis* ((Cav.) Trin. ex Steud.), a perennial grass introduced from Europe, is an aggressive invader of North American wetlands (Saltonstall 2002; Catling and Mitrow 2011). Once established, invasive *P. australis* changes its environment. It produces extensive below and aboveground biomass (eg., Moore et al. 2012, Lei et al. 2019, Yuckin and Rooney, 2019), alters nutrient stocks (e.g., Meyerson et al. 2000, Yuckin and Rooney 2019) and creates a tall, dense canopy that shades out other wetland plants (e.g., Hirtreiter and Potts 2012). Unfortunately, because invasive *P. australis* is so widespread in North American wetlands (Catling and Mitrow 2011; Carson et al. 2018), management of established populations is often restricted to on-going asset-based protection and containment. For example, a survey of 285 U.S. land managers by Martin and Blossey (2013) found managers spent >$4.6 million USD/y on *P. australis* suppression. In Ontario, municipalities reported spending $2.8 million CAD to manage *P. australis* in 2019 alone (Vyn 2019). Given the large amount of public funds directed to invasive *P. australis* suppression, it is critical that the efficacy of management actions is evaluated.

The application of either glyphosate- or imazapyr-containing herbicide is the most common management action applied to *P. australis* in North America (e.g., Martin and Blossey 2013; Hazelton et al. 2014; Hunt et al. 2017). However, glyphosate and imazapyr application to control invasive species in standing water is prohibited in Canada, though and an imazapyr formulation permissible for use in standing water is presently under consideration by the Pest Management Regulatory Authority (Health Canada 2020). Consequently, studies on the efficacy of herbicide in *P. australis* control are nearly all based in the USA. Most studies that used herbicide to control large populations report success in reducing the abundance of *P. australis* (e.g., Bonello and Judd 2019, Rohal et al. 2019a), but eradication is difficult to achieve (e.g.,

Quirion et al. 2017) and most caution that repeated control measures are required to suppress re-growth (e.g., Lombard et al. 2012, Bonello and Judd 2019).

Unfortunately, the record of herbicide-based *P. australis* control in achieving recovery of wetland floristic quality is more mixed. In the absence of other conservation or land management mandates, recovery should target a vegetation community that resembles the community present in equivalent edaphic and hydrologic conditions where *P. australis* never invaded (i.e., the reference condition, *sensu* Stoddard et al. 2006). Some studies report an increase in native vegetation (e.g., Farnsworth and Meyerson, 1999) or floristic quality (e.g., Bonello and Judd 2019) and an increase in similarity to reference vegetation community composition within 3 years (e.g., Zimmerman et al. 2018) after *P. australis* removal using glyphosate. Yet, other studies using glyphosate or imazapyr herbicides do not observe improvements in floristic quality (e.g., Judd and Francoeur 2019) and report that treated vegetation communities do not resemble reference conditions, even four years after herbicide treatment (Rohal et al. 2019a). Given the range of herbicide formulations, marsh types (tidal, riverine, freshwater coastal, etc.), landscape contexts, and invasion histories being compared, it is not yet possible to predict where herbicide treatment will achieve recovery of a desired vegetation community and where it will not. Studies that characterize baseline conditions (e.g., pre-treatment stem densities and floristic diversity), document key covariates like water depth, explicitly define restoration targets and implement experimental controls are needed to advance our understanding of why some projects are more successful than others.

The purpose of our study was to assess the efficacy of invasive *P. australis* control in biodiversity hotspots on the north shore of Lake Erie, including two provincial parks that are

Important Bird Areas, one of which is designated a UNESCO World Biosphere Reserve, and a Ramsar wetland. The work we present here comes from the first large-scale (435 ha) application of glyphosate-based herbicide directly over standing water to control *P. australis* in Canada. This unprecedented action was pursued by provincial managers because of the direct and immediate threat the extensive *P. australis* invasion presented to multiple species at risk, including plants, herptiles, and marsh birds (OMNRF 2017). We use a spatially replicated BACI design with control and treatment plots paired by water depth (10 – 48 cm) to allow us to quantify the influence of water depth on treatment efficacy. First, we assessed how effective the aerial application of a glyphosate-based herbicide was at suppressing *P. australis* when applied over standing water of varying depths. Second, we assessed the initial recovery of vegetation in the first two years post-treatment along the same water depth gradient, comparing recovering vegetation to uninvaded, reference emergent and meadow marsh vegetation communities.

3.2 Materials and Methods

3.2.1 Study Location

Our study took place in two marsh complexes located on the north shore of Lake Erie: Long Point peninsula and Rondeau Provincial Park. These Great Lakes coastal marsh complexes are approximately 165 km apart and are directly connected to their respective bays and sheltered from Lake Erie proper by sand bars (Fig. 3.1). Long Point and Rondeau represent over 70% of the remaining intact wetlands on the north shore of Lake Erie, and as such provide habitat for rare and at-risk species (Ball et al. 2003). These ecosystems, however, are threatened by invasive *Phragmites australis* ssp. *australis*, likely haplotype M (Wilcox et al. 2003). Of the 217 species designated as Species at Risk under Ontario's Endangered Species Act (S.O. 2007 c.6), 25% are

directly threatened by invasive *P. australis*, including 24 species of vascular plants (Bickerton 2015).

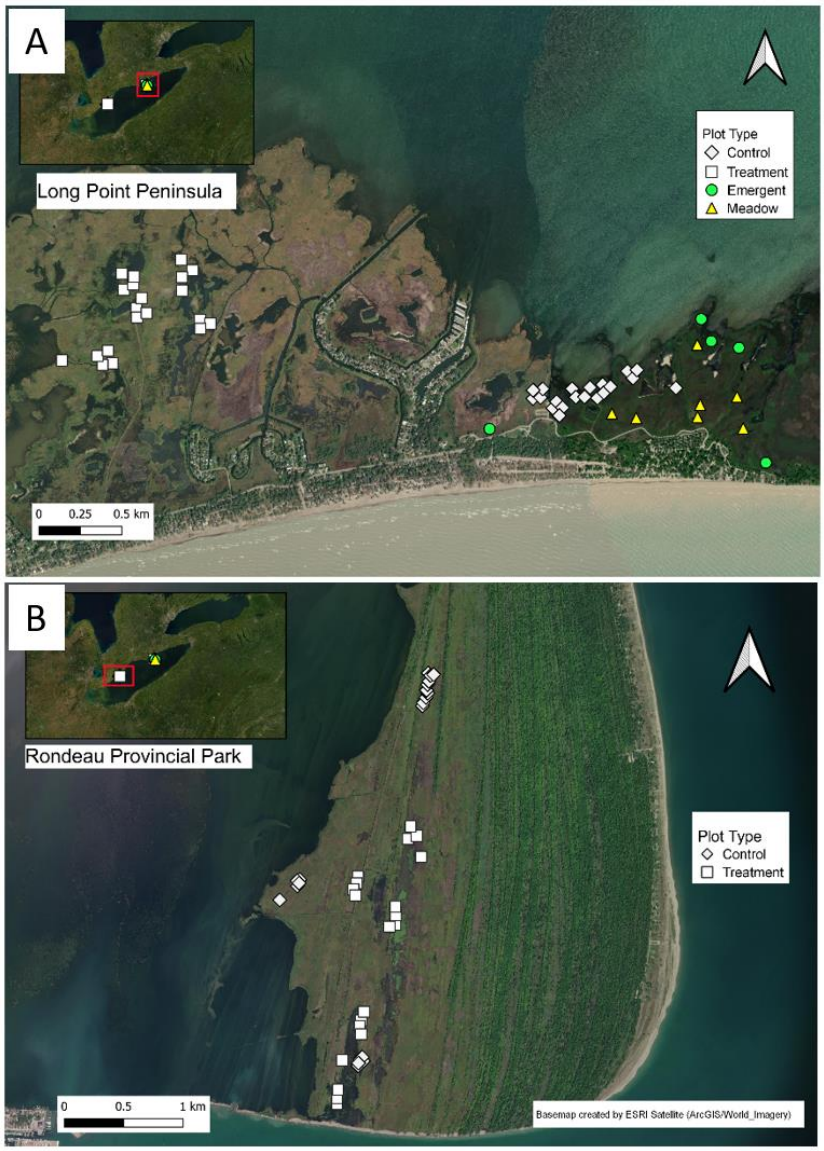

Figure 3.1 Placement of control and treatment 1 m^{-2} plots to assess efficacy of glyphosate-based herbicide treatment on *P. australis* in the western portion of Long Point peninsula (A) and Rondeau Provincial Park (B), located on the northern shore of Lake Erie. Reference condition plots were established in Long Point (A) in meadow marsh and emergent marsh communities. Each reference condition point consists of three independent 1 m^{-2} plots spaced a minimum of 10 m apart.

3.2.2 Phragmites australis aerial treatment

The Ontario Ministry of Natural Resources and Forestry (OMNRF), in partnership with the Nature Conservancy of Canada (NCC), and the Ontario Ministry of Environment, Conservation and Parks (OMECP) obtained an Emergency Registration (#32356) under the Pest Control Products Act from Health Canada's Pest Management Regulation Authority and a provincial Permit to Perform an Aquatic Extermination of invasive *P. australis* in standing water. In September 2016, 335 ha of *P. australis* was treated by licensed contractors in the western portion of Long Point peninsula, and 100 ha was treated in Rondeau Provincial Park. The treatment used glyphosate (Roundup® Custom for Aquatic & Terrestrial Use Liquid Herbicide, Bayer CropScience Inc., Canada), combined with a non-ionic alcohol ethoxylate surfactant (Aquasurf®, Registration Number 32152, Brandt Consolidated Inc., Springfield, Illinois). Using a helicopter for aerial application (Eurocopter A-Star equipped with GPS guidance and Accu-flo boom nozzles), 4210 g acid equivalent (a.e.) glyphosate ha^{-1} as an isopropylamine salt, combined with Aquasurf® non-ionic surfactant at 0.5 L ha^{-1}, was applied at a rate of 8.77 L ha^{-1}, with a total spray mix of 70 L ha^{-1}. We provide details on the concentration of glyphosate in water, sediment and biofilms following herbicide treatment in companion papers (Beecraft and Rooney 2020; Robichaud and Rooney 2020). In February 2017, the treated marsh in Long Point was mowed using a Marsh Master™ or rolled with a drum pulled by an Argo™ track vehicle to knock down standing dead culms of *P. australis*. No mulching took place and mowing vs. rolling depended on equipment availability and site accessibility, with the Marsh Master™ able to reach sites to the north and west that the Argo™ could not. Mechanical secondary treatment did not take place in Rondeau Provincial Park.

3.2.3 Field methods

In August 2016, we established eighty 1 m^2 plots in Long Point peninsula (n = 40) (Fig. 3.1A) and Rondeau Provincial Park (n = 40) (Fig. 3.1B). We then marked plot corners with flagging tape, metal stakes, and a GPS/GNSS unit with sub-meter accuracy (SX Blue II, Geneq Inc., Montreal, PQ, Canada) to ensure the exact locations could be resampled in subsequent years. We situated the plots in dense *P. australis* (> 20 stems m^{-2}) in a stratified-random manner, such that in each marsh treatment (n = 20 per marsh) and control (n = 20 per marsh) plots were paired by water depth, ranging from 10 cm to 48 cm deep (Appendix 2A). This water depth represented the range of standing water depths across which dense invasive *P. australis* occurred in our study area (Fig 3.2). Low density *P. australis* patches were excluded as they were not candidates for herbicide application. In Rondeau Provincial Park, land managers were able to preserve patches to serve as control sites spatially mixed among the treated *P. australis*, whereas in Long Point the entire western region of the marsh was treated, and control sites were in a similar area of marsh about 2 km to the east (Fig. 3.1).

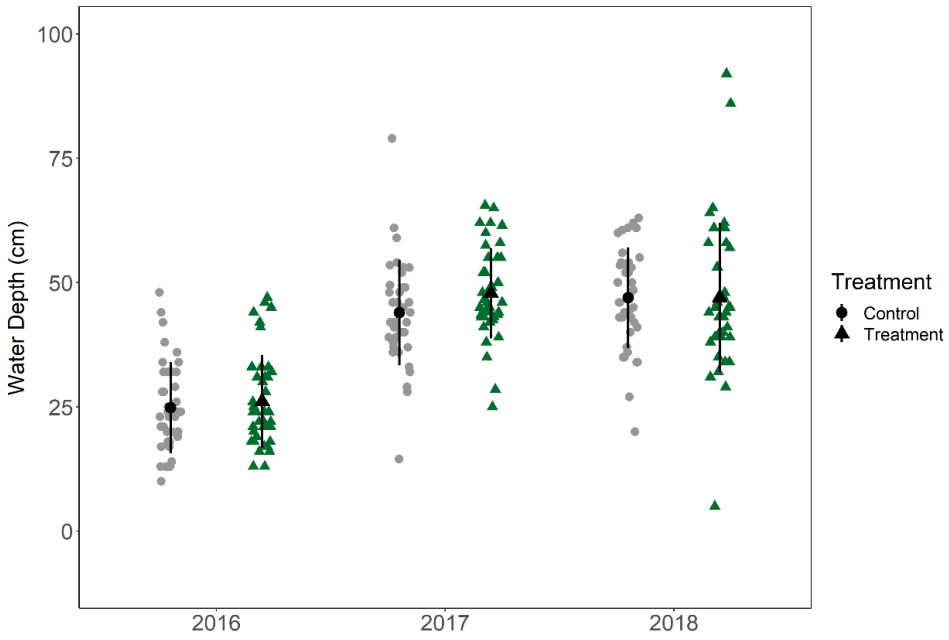

Figure 3.2 Differences in standing water depth between the control and treatment plots among the three years. Plots in Long Point and Rondeau Provincial Park are combined. Figure created with ggplot2 (Whickham 2016).

All plots were surveyed in August 2016, before treatment, and re-surveyed in August 2017 and August 2018: one- and two-years post-herbicide application. Importantly, in Rondeau Provincial Park, one control plot was accidentally sprayed with herbicide: resulting in 39 control and 41 treatment plots. In 2018, a second Rondeau Provincial Park control plot became inaccessible, leaving 38 control and 41 treatment plots. At each plot we measured relevant ecological variables and vegetation community composition. We characterized the vegetation community composition of the plots based on the percent cover of all plant species and non-living cover, such as the litter and standing dead of all species and open water, using a modified Braun-Blanquet cover-abundance method (Wikum and Shanholtzer 1978). Percent cover was considered from a single canopy layer so that each quadrat added up to 100% (± 10%), and

species present at less than 1% cover were recorded as 0.05% to document their presence. All invasive *P. australis* stems, living and dead, were counted in each quadrat. Percent incident light reaching the substrate or water surface was measured using a LI-COR LI-1500 light sensor logger with two LI-190 Quantum sensors that measures photosynthetically active radiation (PAR) in the 400 to 700 nm waveband in µmol m^{-2} s^{-1} which permitted simultaneous measurement of the intensity of incident PAR and PAR passing through the canopy. Precautions were implemented to reduce damage to the plots, including avoiding trampling by having all technicians walk in a single file with one at the lead to find the plot then guide others in. We remained outside of the plot and did measurements by reaching in, taking care to avoid damaging any plants within or around the perimeter of the plot (e.g., no bending stems, stripping leaves, affecting cover or height of plants). Voucher specimens for identification were taken from outside the plot. We then followed our single file path out of the area to minimize additional trampling.

3.2.4 Reference vegetation

In 2017 and 2018 in Long Point, we also characterized the resident vegetation community composition (henceforth "reference condition") along a similar range of water depths (10 - 56 cm) which encompassed meadow marsh (shallow standing water, hummock forming sedges (*Carex* spp.) and grasses (e.g., *Calamagrostis canadensis* ((Michx.) P. Beauv) and emergent marsh (deeper standing water, robust emergent vegetation (e.g., *Typha* spp.)). We established thirty 1 m^{-2} plots in 2017, and twenty-one in 2018, with all plots a minimum of 10 m apart and a maximum of 1.6 km apart (Fig. 3.1). The plots were spread equally between meadow (n = 15) and emergent marsh (n = 15) in 2017, but slightly favored emergent marsh (n = 12) over meadow marsh (n = 9) in 2018 as we discarded four plots that had water depths < 10 cm.

3.3 Statistical Methods

3.3.1 *P. australis* suppression

As a significant interaction effect is the hallmark of an effective treatment in a BACI design, we used two-way ANOVAs (type III SS) with treatment (control or herbicide-treated) and year (2016 - 2018) as fixed factors to test for the effect of herbicide application on total and live invasive *P. australis* stem density. We also applied two-way ANOVAs (type III SS) to test for differences in canopy height (cm), and percent incident light (%) with treatment and year as fixed factors. To meet assumptions of normality in residuals we $\log_{10}$ transformed percent incident light. As Long Point had secondary treatment to reduce standing dead biomass, and Rondeau did not, we also compared total stem density and percent incident light (PAR penetration) in the treatment plots between locations and years (2017 & 2018) with two-way ANOVAs. Where there was a significant effect of a fixed factor, and not the interaction term, we conducted a Tukey's HSD test. All univariate analyses were carried out using the *car* package (Fox and Weisberg 2019) and *agricolae* package (de Mendiburu 2020) in R v. 3.6.2 (R Core Team 2016).

3.3.2 Efficacy of herbicide along a water depth gradient

To assess how effective glyphosate-based herbicide application was along the water depth gradient we compared live invasive *P. australis* stem density in treatment and control plots one year after treatment (i.e., 2017 data) using an ANCOVA, with treatment as a fixed factor and water-depth as a covariate.

3.3.3 Vegetation community response to treatment

To assess if the vegetation community composition changed in response to herbicide treatment, we conducted a two-way perMANOVA with treatment and year (2016 – 2018) as

fixed factors. We applied a general relativization so that percent cover added up to 100% for each plot and removed any species that occurred in two or fewer plots (10 species were removed). Because the number of control and treatment plots was unequal, we used random sampling with replacement that was stratified based on treatment and year. Thirty-eight plots from each treatment and year combination were randomly chosen for each perMANOVA iteration, which we ran 500 times, and we then took the average of each test statistic. This analysis was performed using PC-ORD 7 (McCune and Mefford 2015).

To visualize vegetation composition changes in the treatment plots from 2016 to 2018 we conducted a non-metric multidimensional scaling (NMDS) ordination using a Bray-Curtis dissimilarity matrix. We combined the vegetation composition data from the control and herbicide-treatment plots with the data from the reference condition plots, which we coded as either meadow marsh or emergent marsh based on water depth and dominant vegetation. We then conducted a general relativization, so that each plot summed to 100%, and removed any species that had two or fewer occurrences in the dataset (17 total). This analysis was performed using PC-ORD 7 (McCune and Mefford 2015).

3.4 Results and Discussion

3.4.1 Suppression of invasive *P. australis.*

For every variable related to invasive *P. australis* there was a significant interaction between treatment and year, indicating that herbicide successfully suppressed invasive *P. australis* in treated plots (Fig. 3.3; Appendix 2B). In 2017, we observed a 99.7% reduction in live *P. australis* stem density (per m^{-2}) in treated plots compared to control plots: on average there were 0.1 (std. = 0.6) live invasive *P. australis* stems m^{-2} in treatment plots compared to 36.8 (std. = 15.3) live invasive *P. australis* stems m^{-2} in control plots (Appendix 2C). In 2018,

there was a 94.7% decrease in live *P. australis* stem density in treated plots compared to control plots: an average of 1.5 (std. = 5.6) live stems m^{-2} in treatment plots and 29.8 (std. = 12.3) live stems m^{-2} in control plots (Fig. 3.3A). The suppression efficacy (e.g., stem density reduction) reported by studies of glyphosate-based *P. australis* management varies quite substantially from lows of 50-60% (e.g., Farnsworth and Meyerson 1999; Ailstock et al. 2001) to highs of > 90% (e.g., Derr 2008; Zimmerman et al. 2018). As such, our results are on the high end of reported suppression efficacy.

The first year after treatment, only one of forty treated plots had live *P. australis*. This plot contained four live ramets but expanded seven-fold to 28 ramets the following year. This is equivalent to the live stem density of untreated plots in 2018 (average = 29.8 live *P. australis* stems m^{-2}; std. = 12.3), illustrating how quickly *P. australis* re-colonization can occur. In the second year after treatment, *P. australis* had recolonized four additional plots, at densities from 1 to 22 live *P. australis* stems m^{-2} (Fig. 3.3A). This rapid expansion and re-invasion from small remnants of *P. australis* mirrors the results of long-term monitoring studies (e.g., Lombard et al. 2012; Quirion et al. 2017). For example, after glyphosate was used to suppress *P. australis*, 13.6% of sites exhibited re-growth the following year (Quirion et al. 2017). It is important to recognize that *P. australis* management typically entails two distinct phases. An initial large-scale treatment with herbicide reduces the extent and density of established *P. australis*, achieving the objective of broad 'suppression.' Afterward, management enters a second 'containment' stage, where follow-up spot treatment is required as part of routine maintenance. Quirion et al. (2017) determined continued containment and spot treatment can keep maintenance costs low, as the probability of re-invasion significantly decreases as invasive *P. australis*-free duration increase, with no re-growth documented after four years of consecutive

absence (Quirion et al. 2017). This emphasizes the importance of long-term monitoring and appropriate project budgeting for follow-up control to prevent re-establishment of invasive *P. australis*. However, monitoring for the potential accumulation of herbicide residues under management strategies of long-term herbicide application should also be conducted, as glyphosate used in *P. australis* treatment is known to accumulate in soils (Myers et al. 2016; Robichaud and Rooney 2020), plant litter (Sesin et al. 2019), and wetland biofilms (Beecraft and Rooney 2020).

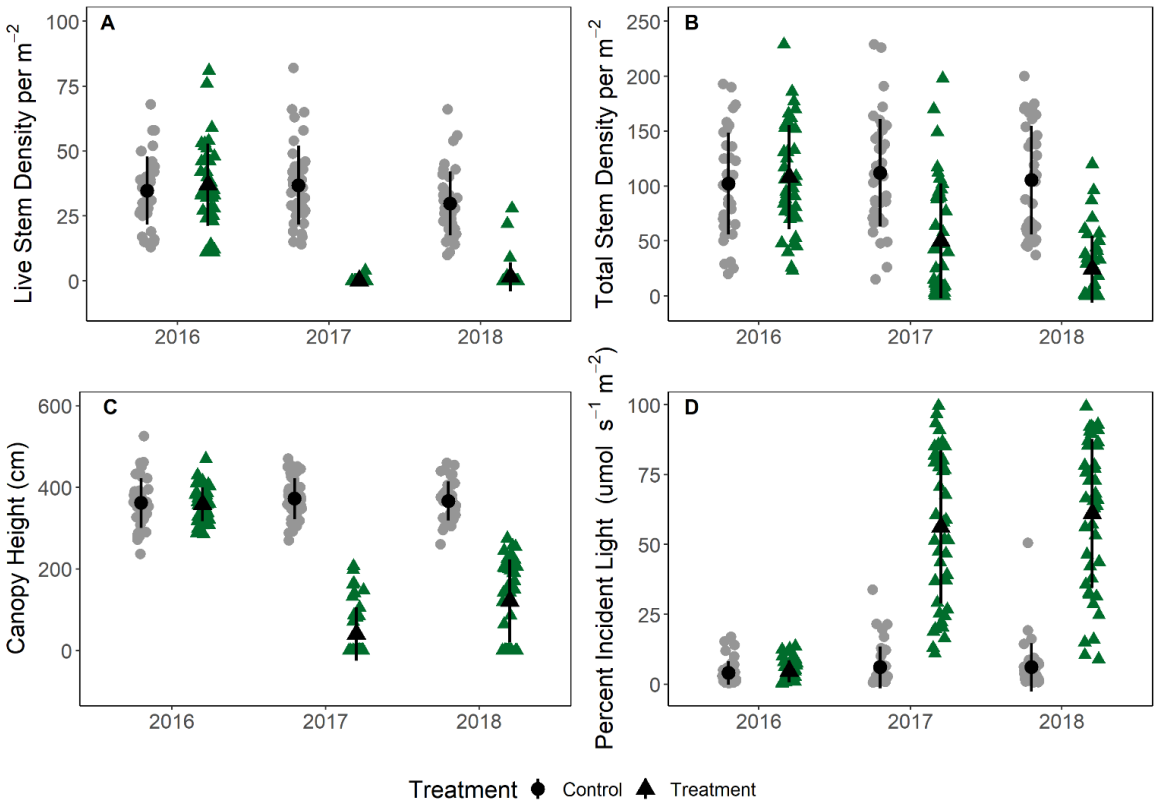

Figure 3.3 There was a significant interaction between treatment type and year for all variables related to *P. australis* suppression: live *P. australis* stems/m^2 (A), total *P. australis* stems/m^2 (B), canopy height (cm) (C), and percent incident light reaching substrate (D). This represents the clear effect of glyphosate-based herbicide at removing *P. australis* from targeted areas. Error bars represent the standard deviation. Created with ggplot2 (Whickham 2016).

There was no difference in live stem density between Long Point, where secondary treatment occurred, and Rondeau Provincial Park, where it did not, after treatment (two-way ANOVA $F_{1,78} \leq 0.88$, $p \geq 0.350$; Appendix 2D). However, there were more standing total stems in Rondeau compared to Long Point after treatment occurred (two-way ANOVA $F_{1,78} = 4.29$, $p = 0.042$). The percent of PAR reaching the sediment also exhibited a significant interaction between location and year (two-way ANOVA $F_{1,78} = 8.46$, $p = 0.005$). The mowing and rolling that took place in Long Point the winter after herbicide application reduced the density of standing dead culms (2017 average 14.0 total stems m^{-2}, std. = 21.5), permitting greater light penetration (68.8% (std. = 26.2%) of PAR reached the substrate in treated plots). In contrast, an average 87.6 total stems m^{-2} (std. = 49.1) remained standing in treated plots in Rondeau, permitting only 44.3% (std. = 23.2%) of incident PAR to pass through the canopy in 2017. Yet total stem densities continued to drop in 2018 (average 2.8 total stems m^{-2} (std. = 7.16) in Long Point and 44.8 total stems m^{-2} (std. = 30.9) in Rondeau) and light penetration reached equivalent levels in 2018, with an average of 56.2% penetration (std. = 28.1%) in Long Point and 66.8% penetration (std. = 25.0%) in Rondeau. Increasing light penetration is likely important to native species recovery, as light penetration can encourage greater seedling establishment (Michinton et al. 2006). Secondary treatment that knocks litter of all species into the water may also speed litter decomposition (Völllm and Tanneberger 2014, Yuckin 2018), further enhancing seedling emergence to facilitate passive restoration of treated areas. However, where secondary treatment to mow or roll is not possible, our results suggest standing dead culm densities and light penetration approach equivalent levels within two years in Great Lakes coastal marsh.

3.4.2 Efficacy of herbicide along a water depth gradient

We observed no effect of water depth on the efficacy of invasive *P. australis* suppression (ANCOVA $F_{1,77} = 0.08$, $p = 0.784$; Appendix 2E), nor was there an interaction between herbicide treatment and water depth (ANCOVA $F_{1,77} = 0.04$, $p = 0.836$), indicating that glyphosate was equally effective across the water depth gradient along which dense invasive *P. australis* occurred (10 – 48 cm). Whereas in semiarid regions, drier sites may result in less successful herbicide-based *P. australis* suppression, as water stress limits the translocation of the herbicide in the plant (Rohal et al. 2019a), in our study area water depth does not affect how well the herbicide works as water depth does not inhibit adsorption by plant leaves and translocation into rhizomes.

3.4.3 Vegetation community response

The vegetation community was likely influenced by flooding, as Lake Erie water levels have been high since 2016 (DFO 2019). Prolonged high water can negatively impact marsh vegetation (van der Valk 2005), and recent work by Keddy and Campbell (2019) suggests that four consecutive years of flooding is enough to drown marsh plants in Lake Erie coastal marshes. Prolonged flooding also limits seedbank regeneration (Keddy and Reznicek 1986). Meadow marsh is less flood tolerant than other vegetation communities and is typically among the most species-rich community in coastal marshes (Reznicek and Catling 1989; Keddy and Campbell 2019). Yet maintaining floooding over 30 cm can prevent *P. australis* seedling emergence (Norris et al. 2002, Baldwin et al. 2010). As such, it is likely that the high Lake Erie water levels after initial herbicide treatment have aided the suppression of invasive *P. australis*, while limiting native vegetation recovery.

Species richness did not differ between control or herbicide treatments in either year (two-way ANOVA F2,233 = 2.87, p = 0.059), nor between treatments (two-way ANOVA $F_{1,233}$ = 0.37, p = 0.544) or years (two-way ANOVA $F_{2,233}$ = 3.01, p = 0.051). Despite this, the vegetation community composition in the plots that were treated with herbicide were compositionally different than the control plots following herbicide treatment (perMANOVA, pseudo-$F_{2, 222}$ = 44.77 (std. = 5.33), p = 0.001 (std. < 0.001); Appendix 2F). The final NMDS ordination was a 3-dimensional solution, with an instability of < 0.0001 after 121 iterations, and a stress of 0.0894 (Fig. 3.4). The proportion of total variance explained was 0.931 (axis 1: 0.618, axis 2: 0.213, axis 3: 0.100). All species and cover class vectors and their correlations with site scores are presented in Appendix 2G. The differences along NMDS axis 1 are driven primarily by *P. australis* abundance. In 2016, before treatment occurred, both control and treatment plots exhibited high *P. australis* abundance. However, one year after herbicide treatment, in 2017, the treatment plots create a new space in the ordination defined by a vegetation community that contains no invasive *P. australis* and continue to occupy this space two years after treatment, in 2018.

The reference vegetation plots separate into two unique vegetation communities, meadow marsh and emergent marsh, along Axis 3 (Fig. 3.4). Meadow marsh was more diverse than emergent marsh and was characterized by *Calamagrostis canadensis* ((Michx.) P. Beauv.) a mix of sedges (e.g., *Carex aquatilis* (Wahlenb.) and *Carex lasiocarpa* (Ehrh.)) and other herbaceous vegetation (e.g., *Cladium mariscoides* ((Muhl.) Torr.)). In contrast, emergent marsh is characterized by high *Typha* spp. abundance, the majority of which is likely the hybrid *Typha* x *glauca* (Godr. (Pro sp.)), though positive identification based on morphology can be challenging in the field. Treated plots fall in the middle of these two reference communities, indicating they

are like, but do not exhibit the same composition as, reference communities one- and two- years after treatment.

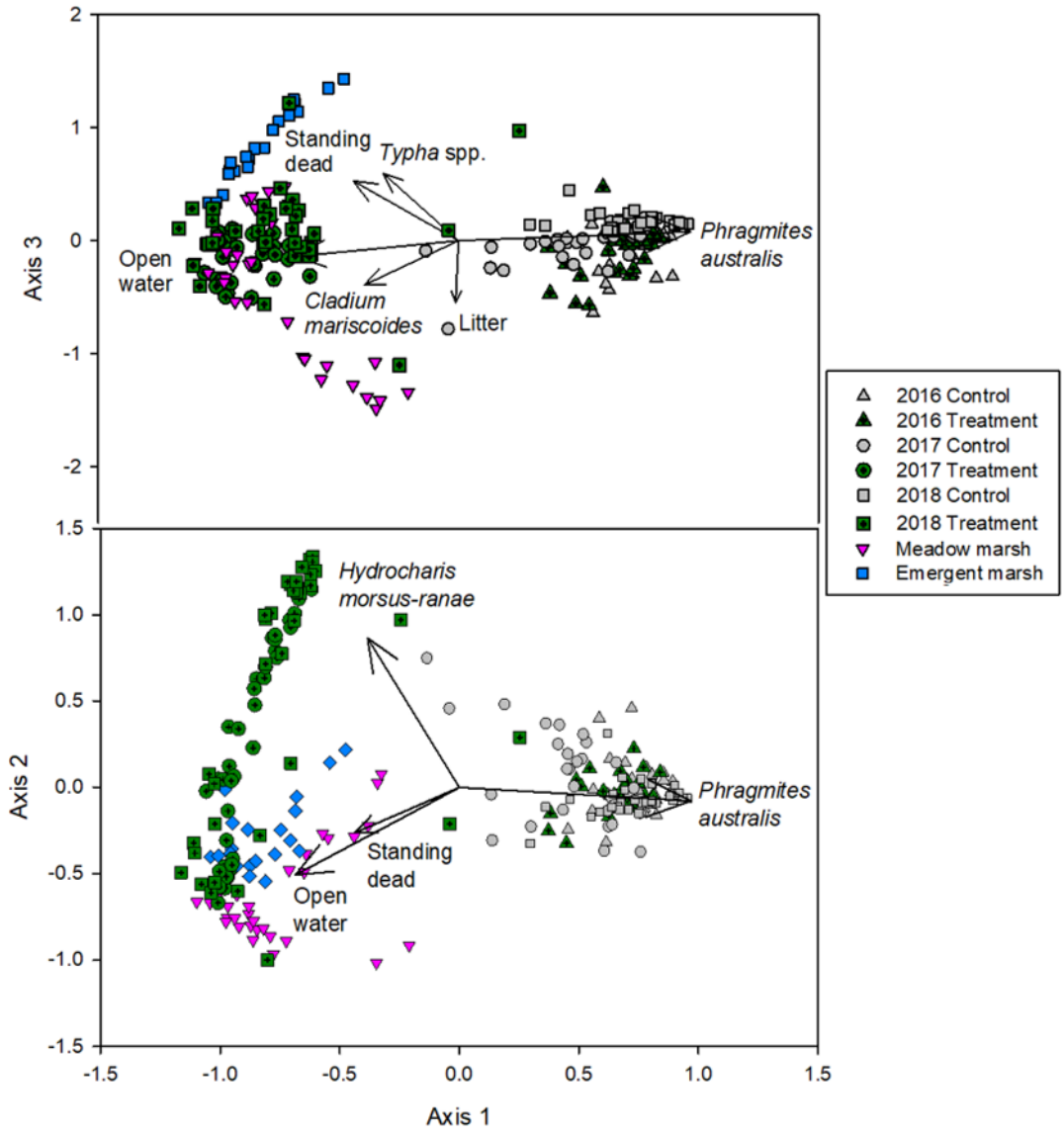

Figure 3.4 The final 3D NMDS ordination solution, using Bray-Curtis dissimilarity matrix. Control and treatment plots were measured in 2016, before glyphosate-based herbicide treatment occurred, and in 2017 and 2018. Reference plots were sampled in 2017 and 2018. Black vectors represent reasonably correlated ($r^2 \geq 0.150$) cover classes.

After two years, more than half of the treatment plots had a community composition that was distinct from the reference condition plots and the control plots. Axis 2 explains the community composition of the treatment plots which either have a high abundance of *Hydrocharis morsus-ranae* (L.), or open water and emergent vegetation (e.g., *Typha* spp.). – *Hydrocharis morsus-ranae* is a small, free-floating aquatic plant native to Europe, Asia, and Africa that was introduced in Canada in 1932 (Catling et al. 2003). *Hydrocharis morsus-ranae* was present in both Long Point and Rondeau before treatment and often co-occurred at low coverage with invasive *P. australis.* The average percent cover of *H. morsus-ranae* in treatment plots before they were treated was 1.73% (std = 2.86%). Robust emergent vegetation has been demonstrated to facilitate the establishment of *H. morsus-ranae* in Great Lakes wetlands by reducing wave action and wind energy (Monks et al. 2019). Yet, in our study the removal of *P. australis* certainly led to an increase in *H. morsus-ranae* cover, increasing to 33.6% (std = 29.8%) one year after and 48% (std = 38.7%) two-years after *P. australis* treatment.

In situations of secondary invasion, we must address whether the secondary invader exerts less negative influence on the invaded ecosystem than the original. If so, the secondary invasion may represent a more desirable outcome than the status quo, even if it does not achieve the restoration of native vegetation diversity. Unfortunately, less has been published about the effects of *H. morsus-ranae* invasion on freshwater wetlands in North America than has been published about *P. australis*. A recent review of *H. morsus-ranae* in North America by Zhu et al. (2018) concluded that dense mats created by *H. morsus-ranae* can have “profound negative” effects on native aquatic plant diversity, excluding important native species, such as the carnivorous *Utricularia vulgaris* (Catling et al 1988). Though a more recent study concluded that *H. morsus-ranae* did not have a negative effect on native plant species richness in Ontario

wetlands (Houlahan and Findlay 2004). Its effects on native plant diversity may be debated, but *H. morsus-ranae* invasion does seem to influence the invertebrate community. Apart from chironomids, which Zhu et al. (2015) concluded were more abundant in *H. morsus-ranae* invaded areas, invertebrate richness and abundance were reduced by *H. morsus-ranae* (Catling et al. 2003; Zhu et al. 2015). More studies on invertebrates and wetland food webs are warranted before we conclude that *H. morsus-ranae* is less harmful than *P. australis*. Of note, recent high water-levels in Lake Erie (DFO 2019) have likely facilitated *H. morsus-ranae* by providing more open water habitat and inhibiting seedbank emergence. Lower lake-levels could reduce the prevalence of this secondary invader in marsh where *P. australis* has been treated with herbicide.

*S*uppression is not only about controlling stem density or footprint of *P. australis*, but also requires managers to account for propagule pressure to limit reinvasion (e.g., Rohal et al. 2019a). Where *P. australis* is abundant, rhizomes and seeds contribute to the establishment or spread of populations at short- and long- distances, respectively (Albert et al. 2015), with seed dispersal being recorded up to 500 m (McCormick et al. 2016). Thus, without coordination at a landscape level, it is likely *P. australis* propagules from surrounding sources will reach the treated area and initiate reinvasion.

Reinvasion and secondary invasions by non-native species present a challenge in restoration (Kettenring and Adams 2011, Pearson et al. 2016). Non-native species are common in restored wetlands, and when coupled with a lack of native propagules, can be a reason restoration projects do not meet their targets (Matthews and Spyreas 2010; Bonello and Judd 2020). Our study is not the first to report a secondary invasion following *P. australis* suppression: herbicide-treatment of *P. australis* invaded Great Lakes coastal marshes in Detroit

resulted in dense populations of *H. morsus-ranae* (Judd and Francoeur 2019). Legacy effects because of established *P. australis,* such as alterations to nutrients (Yuckin and Rooney 2019, D'Antonio and Meyerson 2002) and litter production (e.g., Holdredge and Bertness 2011), and shifting environmental conditions (Pearson et al. 2016) in and around Lake Erie, for example nutrient pollution (e.g., Mohamed et al. 2019) and climate change (e.g., Zhang et al. 2020), increase the likelihood of reinvasion and secondary invasions.

With the current distribution and level of establishment of invasive *P. australis* in North America, most control projects must focus on suppression, containment, and asset protection rather than aim for complete eradication. As invaded area increases, it becomes increasingly difficult to achieve eradication (Quirion et al. 2017) or to recover native vegetation communities (Rohal et al. 2019b). While eradication may not be possible, ecological benefits can be achieved through continuous maintenance and containment at relatively low costs (e.g., Turner and Warren 2003). Annual applications of herbicide to smaller areas (e.g., hand-treating 5% of an invasive population) can effectively reduce *P. australis* populations over time (Turner and Warren 2003), keeping them at an "ecologically benign" level. For example, wetland bird communities responded positively to *P. australis* when it occupied a small portion of the marsh in Long Point, with high species richness along the edges of stands (Meyer et al. 2010). In contrast, once *P. australis* accounted for nearly 70% of the land cover in these marshes, wetland bird communities using *P. australis* were reduced to a subset of the diverse community using remnant marsh (Robichaud and Rooney, 2017). It is also possible to promote plant species diversity by reducing *P. australis* abundance such that light availability and other ecosystem effects (e.g., litter accumulation) do not impede native species establishment (Carlson et al. 2009). Recent assessments have demonstrated marsh birds (Tozer and Mackenzie 2019) and at-

risk plant species (Polowyk 2020) are responding positively to *P. australis* suppression in Long Point. Monitoring the responses of wetland biotic communities to the large-scale suppression of an established invasive species is important as results allow managers to accurately assess the outcomes of control projects.

Long-term monitoring to evaluate potentially harmful contamination is an essential component of herbicide-based invasive species control projects. Over the duration of this project, the concentrations of herbicide in the water and soil in Long Point and Rondeau never approached 0.8 ppm, the concentrations deemed concerning for the protection of aquatic life by the Canadian Council of Ministers of the Environment (CCME; Robichaud and Rooney 2021). However, it is important to acknowledge that this project occurred in protected areas where herbicide had not been previously applied. With multiple applications, glyphosate can accumulate in sediment (e.g., Battaglin et al. 2014, Myers et al. 2016) or wetland vegetation (Sesin et al. 2019), and there are potential sublethal effects of glyphosate in aquatic systems (e.g., Vera et al. 2010; Beecraft and Rooney 2020). Counter-intuitively, small, repeated herbicide applications may reduce overall herbicide use (Turner and Warren 2003), keeping costs and contamination risks low by avoiding the need for large-scale retreatment after reinvasion (Quirion et al. 2017). Follow-up treatment and monitoring thus require long term budget allocations but given that 25% of Ontario's species at risk are threatened by *P. australis* invasion (Bickerton 2015), it is a worthwhile investment.

The question remains whether native species will be capable of recolonizing and persisting in areas where invasive *P. australis* has been dominant for over two decades, particularly given continued persistence of *P. australis* propagule sources in the local seed bank

and the surrounding landscape. Passive restoration is a technique commonly used in wetlands due to the persistence of native species' seeds (Galatowitsch 2018). It has the benefits of being relatively inexpensive and rapid (Gornish et al. 2017) as it relies on propagules that are present within, or can easily reach, the target area. As such, the species present in seed banks are an important determinant of the success of passive restoration. However, seed banks in areas that have been invaded typically have a lower native species density and richness while containing a higher richness or abundance of non-native species (Gioria et al. 2014). The diversity of native plants in the seed banks of *P. australis* invaded sites appears to vary depending on the system and history of invasion, though some studies suggest it is sufficient for passive restoration (e.g., Howell 2017; Hazelton et al. 2018). For example, the seed bank in *P. australis* stands in brackish tidal wetlands was more species rich than the standing vegetation suggested (Baldwin et al. 2010). These seed banks do contain viable *P. australis* seeds, however, and stands that persist within 500 m of the treated area can be a source of viable seeds which would lead to re-invasion (Ailstock et al. 2001, McCormick et al. 2016). Removing the stress or pressure of an invasive species may not be enough to result in a native community that meets restoration goals, including creating a diverse plant community capable of resisting reinvasion by *P. australis* (Peter and Burdick 2010).

Active revegetation, or re-seeding treated areas, provides an opportunity for managers to promote native plants with attributes that can resist further invasion (Simmons 2005; Byun et al. 2018), though seeding wetland species is not without challenges (e.g., England 2019). Invasive species are typically early colonizers of disturbance, making it important for managers to identify a) the legacy of the previously established invasive species and b) which native species can tolerate local conditions and invasion pressure (Hess et al. 2019). Trait-based restoration

approaches may be applied to select native species that can tolerate the environmental conditions of the site and influence biotic interactions (Laughlin 2014). This approach requires a clear understanding of the dynamics of the system, and the fitness and niche differences between native species and non-native species (MacDougall et al. 2009), to select appropriate candidate species for seeding. Seeding can lead to more native species cover and higher species richness (e.g., Kettenring and Tarsa 2020) and may permit cultivation of species to increase biotic resistance to reinvasion (Byun et al. 2018). Whether or not managers decide to actively seed or passively allow propagules to reach a site is a choice that is ultimately dependent on their goals, though there is a growing body of literature that suggests planned revegetation could be an effective way to prevent the re-establishment of non-native species (Byun et al. 2018).

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4 Macroinvertebrate assemblages in *Phragmites australis* stands, herbicide-treated areas, and remnant coastal marsh.

4.1 Introduction

Invasive plant species pose a global threat to biological communities. Invasive plants can alter biogeochemical cycling regimes (Vilà et al. 2011), hydrology (Le Maitre 2004), and habitat structure (Crooks 2002) which negatively impacts native species. Invasive plants are often associated with degraded environments (e.g., Früh et al. 2012) and benefit from stressors, such as pollution and altered disturbance regimes, while also driving declines in native species and further degradation (e.g., MacDougall and Turkington 2005, Bauer 2012). Ecological restoration of degraded environments is often hampered by the presence of established invasive species (D'Antonio and Meyerson 2002). Typically, the first step in restoring these degraded ecosystems is the control or removal of an invasive plant, with the intention that the native biological community will reassemble (Weidlich et al. 2020). However, often studies following the removal of an invasive plant species only measure variables related to the vegetation community recovery (e.g., Hazelton et al. 2014). To fully assess the effects of invasive species control and restoration efforts it is essential to characterize the biological communities present in invaded vegetation and in remnant, uninvaded vegetation.

Wetlands are particularly vulnerable to invasive plants due to their dynamic environmental conditions (Zedler and Kercher 2004) and ongoing global loss and degradation (e.g., Brinson and Malvárez 2021). In North America, Lake Erie has approximately 19,330 ha of coastal wetlands which are some of the most diverse and species rich in the Laurentian Great Lakes region

(Herdendorf 1992, Ball et al. 2003). This diversity includes complex macroinvertebrate communities consisting of aquatic, semiaquatic, and terrestrial taxa (Batzer et al. 1999). Macroinvertebrates are often the most diverse and abundant fauna within a wetland and provide an essential link between primary producers (e.g., plants, algae) and higher trophic levels (Batzer et al. 1999). Wetland macroinvertebrates are resilient to natural variations in their environment but are likely to respond strongly to dramatic changes (Batzer 2013). In Lake Erie coastal wetlands, this poses an interesting hypothesis as wetlands are changing dramatically due to invasive plant species and invasive plant species control actions.

Invasive *Phragmites australis* subsp. *australis* (European common reed [(Cav.) Trin. Ex Steud.]) is a perennial wetland grass that has established itself throughout Great Lake wetlands (e.g., Wilcox 2012). *Phragmites australis* rapidly expanded in Lake Erie beginning in the late-1990s after periods of low water levels (Tulbure and Johnston 2010, Wilcox 2012). Within two decades *P. australis* had created monocultures in formerly diverse wetland systems including the Long Point peninsula, a World Biosphere Reserve on Lake Erie (Wilcox et al. 2003, Jung et al. 2017). Monocultures of *P. australis* create dense stands which produce large amounts of litter (e.g., Yuckin and Rooney 2019), decrease wetland avian diversity by excluding species of conservation concern (e.g., Robichaud and Rooney 2017), and are detrimental to endangered reptiles (e.g., Markle and Chow-Fraser 2018) and amphibians (e.g., Greenberg and Green 2013). The documented negative effects of invasive *P. australis* have led to on-going efforts to control populations and restore native wetland vegetation in North America (e.g., Hazelton et al. 2014). This includes the recent large-scale application of a glyphosate-based herbicide directly over standing water in the marshes of Long Point, a thus far unprecedented control action in Canada (Robichaud and Rooney 2021a). Herbicide-application is a common tool used to manage *P.*

australis (e.g., Hazelton et al. 2014), and while glyphosate-based herbicide degrades rapidly to non-toxic levels (Robichaud and Rooney 2021b), large macrophyte die-offs can affect native fauna (e.g., Linz et al. 1999).

The response of macroinvertebrate communities to *P. australis* invasion, and *P. australis* control, are not as clear as the response of other wetland fauna. In a comparison of benthic macroinvertebrate densities among invasive *P. australis, Typha angustifolia,* and native flora on the southern shore of Lake Erie, macroinvertebrate diversity increased with *P. australis* cover and density was similar among the vegetation types (Holomuzki and Klarer 2010). Using soil cores and litter packs to sample macroinvertebrates in brackish marshes, Angradi et al. (2001) found that macroinvertebrate densities and richness were higher in native *Spartina* vegetation than *P. australis.* In salt marshes in the lower Connecticut River estuary, sediment, litter, and pitfall trap samples indicated that macrofaunal densities were lower in *P. australis* invaded marsh but taxon richness was higher (Talley and Levin 2001). Another study in the lower Connecticut River compared macroinvertebrates captured in litter bags and pit fall traps among treated (herbicide and mowing) *P. australis,* untreated *P. australis* and *Typha angustifolia* (Fell et al. 2006). The densities of macroinvertebrates from pit fall traps were similar among all three habitats, but densities from litter bags were significantly higher in *T. angustifolia* vegetation (Fell et al. 2006). In a Lake Erie marsh, benthic macroinvertebrate diversity was similar among herbicide-treated *P. australis,* untreated *P. australis,* and *T. angustifolia,* though densities were higher in both *P. australis* treatments due to gastropods and chironomids (Kulesza et al. 2008). By examining the invertebrate community in the vegetation (e.g., stems and leaves) of a marsh, Gratton and Denno (2005) found that *Phragmites australis* invasion altered the invertebrate community in brackish marshes, reducing the densities of dominant marsh spiders and

supporting more detritivores like Collembola and chironomids. *Phragmites australis* control using a glyphosate-based herbicide, however, resulted in the recovery of the invertebrate community associated with the native vegetation in less than five years (Gratton and Denno 2005).

Most studies examining macroinvertebrate communities in *P. australis* or in areas where *P. australis* has been treated focus on benthic macroinvertebrates, which may limit inferences regarding changes to invertebrate assemblages caused by an invasive plant (Harvey et al. 2014). The aquatic stage of many macroinvertebrates can be useful in determining the health of an ecosystem as they often have known responses to environmental stressors (Bonada et al. 2006), though there are challenges creating bioindicators for the variety of conditions in Great Lakes coastal wetlands (e.g., Batzer 2013). However, many freshwater invertebrates require access to terrestrial habitat for at least one life cycle stage - the complete life cycle of aquatic invertebrates (egg to adult) can occur once to 3+ times a year, with the aquatic life stage ranging from fewer than two weeks to several years depending on the species (Merritt et al. 2008). Additionally, sampling only aquatic microhabitats (e.g., inundated litter, saturated soil) may miss the terrestrial macroinvertebrates that rely on wetlands (Batzer and Wu 2020). Changes to vegetation by using herbicide can result in increased biomass of emerging invertebrates, as demonstrated in Baker et al. 2014 and Linz et al. 1999, which can influence aquatic-terrestrial linkages or food webs. Therefore, it is important to sample both aquatic invertebrates, either those that will spend the whole summer in the aquatic environment or those that will emerge as adults, and those that use the vegetation community to fully capture changes because of invasion and invasion control.

We conducted a study in the coastal marshes of Long Point, ON, to characterize the invertebrate community structure in *P. australis*-invaded marsh, recently herbicide-treated marsh, and remnant, uninvaded marsh habitat. To capture the variation in macroinvertebrate diversity, we sampled aquatic invertebrates (collecting submerged vegetation) and emerging invertebrates (emergence traps). This comprehensive sampling should capture the aquatic invertebrates, semi-aquatic, and terrestrial invertebrates that rely on wetlands. We addressed three research questions: 1) Do *P. australis* stands have lower densities or taxonomic richness than remnant, uninvaded marsh? 2) Does the macroinvertebrate community in herbicide-treatment areas more closely resemble remnant uninvaded marsh, or *P. australis* invaded marsh? and 3) Does the agreement between the juvenile aquatic invertebrate community composition and the winged terrestrial community composition differ among *P. australis* invaded sites, remnant marsh, and herbicide-treated sites?

4.2 Methods

4.2.1 Site establishment and characterization

Our study took place in Long Point, Ontario, Canada. Located on the north shore of Lake Erie, Long Point is a sandspit peninsula that contains ecologically significant vegetation communities and provides habitat for hundreds of species (Ball et al. 2003). Populations of invasive *Phragmites australis* subsp. *australis* have been growing in Long Point since the late 1990s, displacing resident wetland vegetation communities and replacing them with dense stands of *P. australis* (Wilcox et al. 2003). In 2016, the Ontario Ministry of Resources and Forestry and the Nature Conservancy of Canada treated over 300 ha of *P. australis* in Long Point using a glyphosate-based herbicide followed by rolling or cutting standing dead stems (details in Robichaud and Rooney 2021b).

In 2018, we established sites in three habitat types: *P. australis*-invaded habitat (n = 9), herbicide-treated habitat (n = 9) and remnant, uninvaded marsh vegetation (hereafter "remnant" marsh; n = 9), for a total of 27 (Fig. 4.1). Herbicide-treated sites had been treated with a glyphosate-based herbicide in 2016, and thus were two- years post-treatment during sampling. At each site, we deployed an emergence trap to collect emergent invertebrates, collected an aquatic invertebrate sample, measured water depth, temperature, and canopy height, and characterized the vegetation (details below).

Sites within each habitat type covered the range of water depths across which herbicide application took place. In May 2018 - *P. australis*-invaded sites ranged from 22.3 cm to 77 cm in standing water depth, herbicide-treated sites from 21.7 cm to 51 cm, and remnant marsh from 7 cm – 75 cm (Appendix 3A). This gradient captured an ecotone of remnant marsh vegetation communities, from meadow marsh habitat, characterized by sedges, grasses, and forbs, with shallow standing water (< 30 cm in May) to emergent marsh with deeper standing water and dense stands of cattail (*Typha* spp.). Sites at intermediate depth were characterized by a mix of meadow marsh and emergent marsh vegetation. Before herbicide application, sites to be treated sites had dense *P. australis* stands equivalent to those in *P. australis* invaded sites. After treatment they were typically characterized by open water with abundant submersed and floating aquatic vegetation (e.g., *Utricularia vulgaris, Potomogeton* spp.) and some emergent vegetation (e.g., cattails, rushes) (Robichaud and Rooney 2021a).

Figure 4.1 Emergence trap sites in Long Point, ON, Canada situation in *P. australis* stands, remnant marsh habitat, and herbicide-treated sites. Each site consisted of a modified emergence trap to capture emerging invertebrates from May to July, and sites were sampled once for aquatic invertebrates in May 2018.

4.2.2 Invertebrate capture

At each site, we deployed a modified emergence trap to capture invertebrates over the field season (Appendix 3B). Emergence traps capture invertebrates with an aerial life stage to quantify the biomass of emergent invertebrates per meter squared. Each trap consisted of a capture vessel atop a pyramid structure on a 1 m^2 base and covered in 500 µm mesh netting (following Anderson and Davis, 2013). Traps ranged in height based on the vegetation

community they were deployed in; 1.5 m tall in herbicide-treated and meadow marsh sites and 2 m tall in emergent marsh and *P. australis* sites. When setting up the sites we made a concerted effort not to disrupt the surrounding vegetation so that we would not affect vegetation density or light penetration that could influence emergence timing or success. The capture vessel contained an aqueous solution of 70% ethanol as a preservative. It was collected and replaced approximately every 10 days from deployment until the end of July which resulted in six collections from 20-May-2018 to 23-July-2018. Not every emergence trap was collected each time, typically due to weather affecting the collection vessel. The number of collections is reported in Appendix 3A.

We also quantified the composition of the aquatic macroinvertebrate community at each site between 11 and 15 of May 2018. We used a 0.25 m^2 quadrat, within which we clipped and collected all standing dead litter, submersed, floating, and emergent vegetation and overlying water into a bucket. We then poured the water through a 500 µm mesh sieve, repeating this rinse process at least four times by adding filtered lake water, agitating the vegetation, and passing it through the sieve. The contents of the sieve were transferred to a collection jar and preserved in 70% ethanol. We then sorted through the remaining vegetation in white trays to collect any clinging invertebrates and added these to the collection jar. Note we also took 8 cm deep 5 cm diameter benthic core samples and 10 cm diameter water column samples at each quadrat sampling location but did not include these in analyses because after processing 100% of the water column and 20% of the soil samples they yielded no additional macroinvertebrate taxa, and soil samples were labor intensive to process. For clarity, we refer to emergence trap samples as “emerging invertebrates” and quadrat samples as “aquatic invertebrates” throughout.

4.2.3 Laboratory processing

Samples were sorted to separate macroinvertebrates from debris under a dissecting microscope. Individuals were identified to the lowest taxonomic level that did not require slide mounting, typically Order or Family, using Merritt et al. (2008) and Thorp and Covich (1991). The two deepest sites in remnant and *P. australis*-invaded habitats are not represented in the aquatic invertebrate analyses as they did not have adequate amounts of vegetation for sampling. Therefore, for emerging invertebrate analyses there are nine sites in each habitat type, for aquatic invertebrate samples there are eight sites in remnant and *P. australis* marsh and nine sites in herbicide-treated sites. For the Procrustes analyses, we removed the same two sites from the emerging invertebrate data so there were an even number of sites in the aquatic and emerging invertebrate datasets.

4.2.4 Statistical methods

Emerging invertebrate data were summed across all visits. Taxa with two or fewer occurrence at any station in the emergence trap samples were removed from the emergence trap data and two or fewer occurrences at any station in the aquatic samples was removed from the aquatic invertebrate data. The final taxa included in analyses are reported in Appendix 3C and 3D. For univariate analyses we calculated the density (number of individuals per site), taxonomic richness and Pielou's evenness (J) for aquatic invertebrates and emerging invertebrates using the vegan package (Oksanen et al. 2020) in R v. 4.0.0 (R Core Team 2020).

4.2.5 Univariate analyses

4.2.5.1 Aquatic invertebrates

As the data did not meet the assumptions of a parametric test, we performed permutational general linear models with taxonomic richness, density, and Pielou's evenness as

response variables and habitat type (*P. australis*-invaded, remnant marsh, and herbicide-treated marsh) as a fixed factor. Each test was run with 999 permutations, producing a p-value based on the number of test statistics from randomized runs that are as or more extreme than the actual test statistic. If habitat type was significant, we performed a Tukey's post-hoc test. Analyses were performed using lmPerm (Wheeler and Torchiano 2016) and agricolae (de Mendiburu 2020) in R v. 4.0.0 (R Core Team 2020).

4.2.5.2 Emerging invertebrates

We used a generalized linear mixed model with a Poisson distribution to determine if emerging invertebrate taxonomic richness was predicted by habitat type and conducted general linear mixed models for density and Pielou's evenness. Habitat type was a fixed effect and number of successful collections (Appendix 3A) was a random effect in all models and we determined the conditional and marginal r^2 via the approach specific by Nakagawa and Schielzeth (2013). However, when assessing the linear mixed models, the variance components of the random effect equaled zero, so we removed the random effect and ran density and Pielou's evenness models as permutational general linear models (ANOVA) with habitat type as fixed factors. Mixed models were fit using the lme4 package (Bates et al. 2015), permutational models with lmPerm (Wheeler and Torchiano 2016), and conditional and marginal r^2 values were calculated using the performance package (Lüdecke et al. 2020) in R v. 4.0.0 (R Core Team 2020).

4.2.6 Multivariate analyses

The following multivariate analyses were performed on the aquatic invertebrate and emerging invertebrate datasets separately. First, we used a permutation test (PERMDISP) to

assess differences in multivariate dispersion among the habitat types by measuring the mean distance to a group centroid in multivariate space (Anderson et al. 2006). This test can provide information on beta diversity within each habitat type and determine if data meet the assumptions of homogeneity of variances necessary for a perMANOVA (Warton et al. 2012). To test if the differences between groups were significant, we used a test that permuted the least-square residuals 999 times to generate a distribution to test the F-statistic against (Anderson et al. 2006). As we were also interested in comparing the invertebrate community composition among the habitat types, we performed a perMANOVA (999 permutations) with habitat type as a fixed factor. We also used a non-metric multidimensional scaling (NMDS) ordination to visualize differences in invertebrate community composition among the habitat types. For PERMDISP, perMANOVA, and the NMDS ordination we used community matrices with density data relativized by column maximums to reduce the influence of highly abundant taxa, and Bray-Curtis dissimilarity matrices. PERMDISP, perMANOVA, and NMDS ordinations were all performed using the vegan package (Oksanen et al. 2020) in R v. 4.0.0 (R Core Studios 2020).

4.2.7 Congruence between aquatic and emerging invertebrates

As aquatic macroinvertebrate samples were taken in mid-May 2018, we summed the emergence trap data from 5 June to 23 July 2018 for congruence analyses. We also removed the deepest remnant and *P. australis*-invaded sites from the emergence trap dataset to match the aquatic invertebrate data for a total of 25 sites in each dataset. Because not all taxa captured in the aquatic invertebrate samples have a winged adult stage, only a subset of aquatic macroinvertebrate taxa are expected to be collected in the emerging invertebrate samples. Similarly, emergence traps collected some semi-aquatic taxa that lack an aquatic juvenile state, and so we would not expect to collect them in the aquatic macroinvertebrate samples. We thus

excluded taxa from both datasets that lacked both an aquatic juvenile stage and a winged adult stage. We then ran three Procrustes tests, one for each habitat type, using the protest function in vegan (Oksanen et al. 2020). This test performs a symmetric Procrustes analysis and permutations to estimate the significance of the t-statistic. We report the Procrustes correlation from the non-permuted solution and the p-value after 999 permutations.

4.3 Results

4.3.1 Univariate

4.3.1.1 Aquatic invertebrates

Aquatic invertebrate density was significantly higher (permutational ANOVA $F_{2,22}$ = 7.301, p = 0.004) in herbicide-treated sites (3374.67 individuals/ ¼ m^2 (st. error ± 973.51)) than *P. australis* stands (583.88 individuals/ ¼ m^2 (st. error ± 219.96)) or remnant marsh habitat (444.63 individuals/ ¼ m^2 (st. error ± 56.01)) (Fig. 4.2A). Two sites had very high densities – one with 9,851 individuals/ ¼ m^2 and another with 6,047 individuals/ ¼ m^2 – consisting of high densities of Oligochaetes and Chironomidae. There was no significant difference in taxonomic richness among the three habitat types (permutational ANOVA $F_{2,22}$ = 2.644, p = 0.094; Fig. 4.2B), though Pielou's evenness did differ significantly among the habitat types (permutational ANOVA $F_{2,22}$ = 3.644, p = 0.043) and was, on average, lower in *P. australis* sites (Fig. 4.2C).

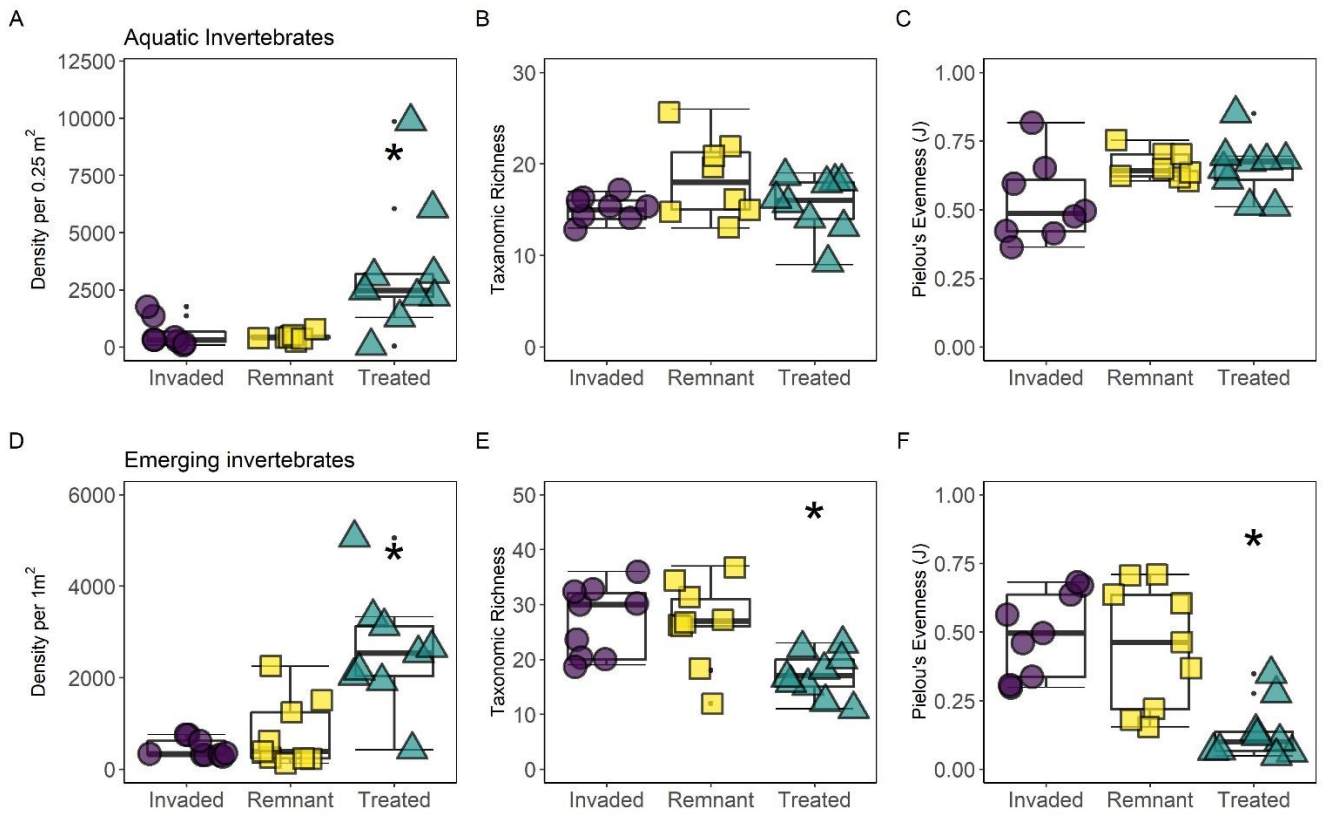

Figure 4.2 Differences in aquatic invertebrate density per 0.25 m^2 (A), taxonomic richness (B), and Pielou's evenness (C) and emerging invertebrate density per m^2 (D), taxonomic richness (E), and Pielou's evenness (F) among habitat types. Boxplot whiskers represent 1.5 * IQR / sqrt(n), and notches represent 25%, 50% and 75% quantiles. Significant differences are indicated with an asterisks (*).

4.3.1.2 Emerging invertebrates

Habitat type predicted emerging invertebrate densities (ANOVA $F_{2,24} = 16.582$, $p < 0.001$), with significantly higher densities in herbicide-treated sites (2580.44 individuals per m^2 (st. error ± 417.38)) compared to *P. australis* stands (449.00 individuals per m^2 (st. error ± 67.21)) and remnant marsh habitat (761.44 individuals per m^2 (st. error ± 246.92)) (Fig. 4.2D).

Habitat type also predicted taxonomic richness, which was significantly lower in herbicide-treated sites (17.11 st. error ± 1.38) compared to *P. australis* stands (27.11 st. error ± 2.14) and remnant marsh (26.44 st. error ± 2.56) (Table 4.1; Fig. 4.2E). Similarly, Pielou's evenness was also significantly lower in herbicide-treated sites (0.14 st. error ± 0.04) compared to *P. australis* stands (0.49 st. error ± 0.05) or remnant marsh (0.45 st. error ± 0.08) (ANOVA $F_{2,24} = 11.914$, $p < 0.001$; Fig. 4.2F).

Table 4.1 Generalized linear mixed model results for emerging invertebrate taxonomic richness. Marginal (r^2m) and conditional (r^2c) coefficients of variation are reported to describe the proportion of variation explained by the fixed effects (marginal) and the entire model (conditional).

	Estimate	Z-statistic	p-value	r^2_m	r^2_c
Intercept	3.084	19.028	<0.001	0.293	0.579
P. australis stands	0.061	0.658	0.510		
Herbicide-treated	-0.369	-3.447	<0.001		

4.3.2 Multivariate

4.3.2.1 Aquatic invertebrates

We detected a total of 29 taxa in *P. australis* stands, 27 taxa in herbicide-treated sites, and 37 taxa in remnant marsh. The mean distance to the group centroid was 0.447 in *P. australis* sites, 0.454 in herbicide-treated sites, and 0.486 in remnant marsh sites indicating beta diversity of aquatic macroinvertebrates was slightly higher in remnant marsh. However, there was no significant difference in the homogeneity of variances among the habitat types (PERMDISP, $F_{2,24} = 0.330$, $p = 0.766$). Consequently, we interpret any statistically significant perMANOVA

results as indicating a difference in community location, rather than community dispersion (Warton et al. 2011).

Aquatic macroinvertebrate community composition differed significantly among the habitat types (perMANOVA pseudo-$F_{2,24} = 4.379$, $p = 0.001$) and each habitat type was significantly different from the other (Bonferroni correct p-value = 0.003 for each comparison). The final NMDS ordination solution was 3-dimensional, with an acceptable final stress of 0.145, and two convergent solutions found after 28 tries, with a non-metric r^2 of 0.979 (Fig. 4.3A). On axis 1 and 2 there was little overlap of the three habitat types. Remnant marsh and herbicide-treated sites separated along axis 1, while *P. australis* sites and herbicide-treated sites separated along axis 2. Remnant marsh was more diverse than either *P. australis* or herbicide-treated sites. Chironomidae (9,756 individuals per ¼ m^2) were associated with herbicide-treated sites and were highly abundant compared to remnant (544 individuals per ¼ m^2) or *P. australis*-invaded (506 individuals per ¼ m^2) sites. Caenidae (small square-gill mayflies) and Leptoceridae (long-horned caddisflies) were also correlated with herbicide-treated sites, as were Hydrozoa (hydrozoans) and Gastropoda (snails). Remnant marsh was characterized by Staphylinidae (rove beetles), Collembola (spring tails), Aranea (spiders), and Hydrophilidae (water scavenger beetles). On axis 1 and 3, herbicide-treated and *P. australis* stands overlapped considerably and had fewer associated taxa than remnant habitat. A few remnant marsh and *P. australis*-invaded shared higher densities of Curculionidae (true weevils) and Coenagrionidae (narrow-winged damselflies) which were orthogonal to sites characterized by Dolichopodidae (long-legged flies). The taxa strongly correlated with remnant marsh habitat on axis 1 were also associated with vegetation litter.

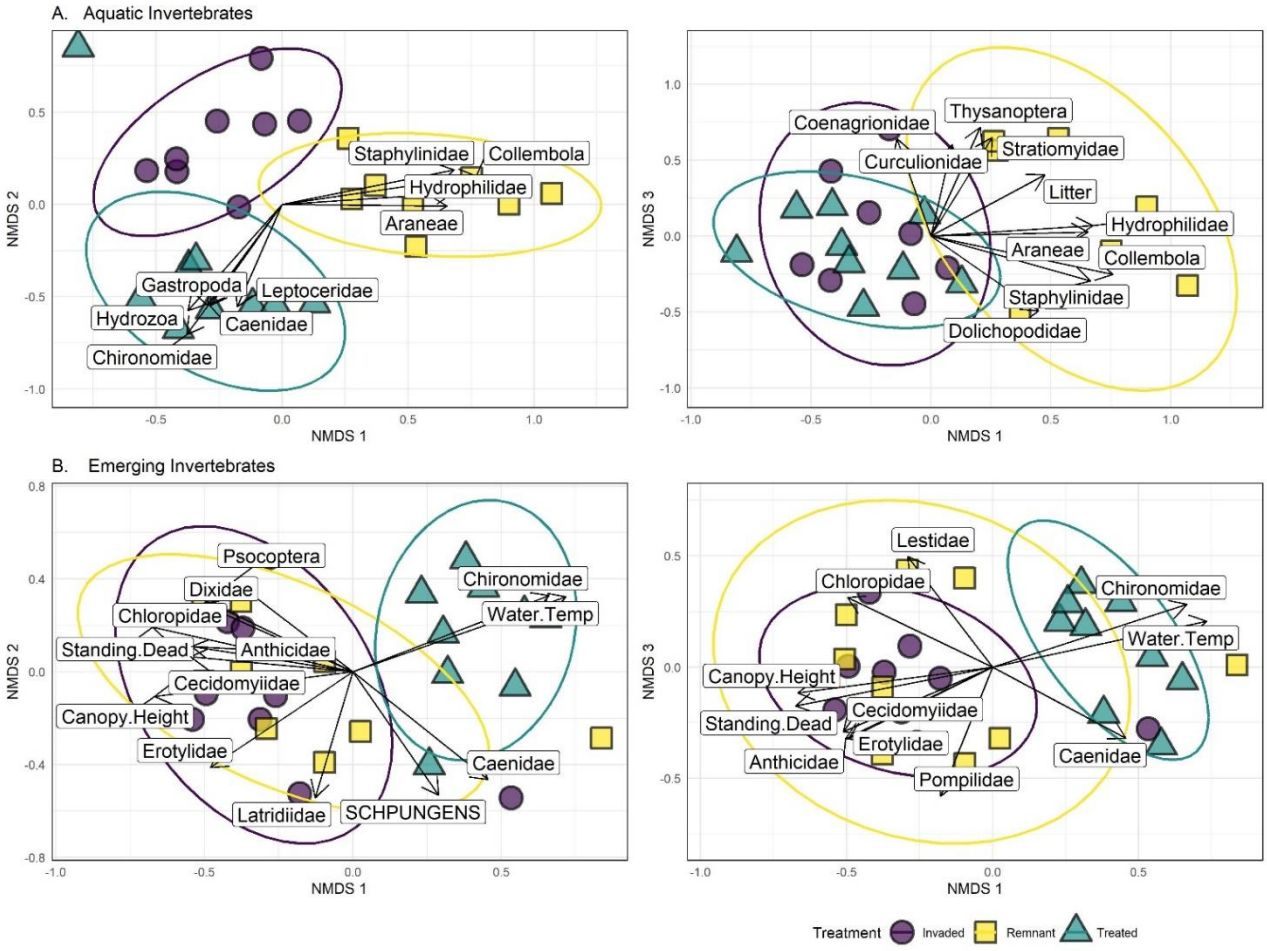

Figure 4.3 NMDS ordination solutions of aquatic invertebrates (A) and emerging invertebrates (B) with reasonably correlated taxa (r2 > 0.30) included as vectors and 90% CI ellipses.

4.3.2.2 Emerging invertebrates

We detected a total of 68 taxa in *P. australis* stands, 43 taxa in herbicide-treated sites, and 66 taxa in remnant marsh. The mean distance to the group centroid was 0.506 in *P. australis* sites, 0.492 in herbicide-treated sites, and 0.541 in remnant marsh sites indicating beta diversity was slightly higher in remnant marsh and lowest in herbicide-treated sites. As with the aquatic invertebrates, there was no significant difference in the homogeneity of variances among the

treatment types (PERMDISP $F_{2,24} = 0.8156$, $p = 0.444$), and as such we interpret the results to indicate a difference in community location rather than dispersion (Warton et al. 2012). The perMANOVA results indicate there was a significant difference in the emerging invertebrate communities among the habitat types (perMANOVA pseudo-$F_{2,24} = 2.941$, $p = 0.001$). Pairwise comparisons determined that remnant marsh and *P. australis*-invaded sites were not significantly different from one another (Bonferroni corrected p-value = 0.067) while the invertebrate community in herbicide-treated sites was significantly different from the community in *P. australis*-invaded sites (Bonferroni corrected p-value = 0.002) and in remnant marsh sites (Bonferroni corrected p-value = 0.001).

The final NMDS ordination was a 3D solution with a stress of 0.159 after 81 iterations and a non-metric r^2 of 0.975 (Fig. 4.3B). There was considerable overlap in the invertebrate community composition between *P. australis* and remnant marsh on all three axes, while herbicide-treated sites were separated from the other habitats along axis 1. Chironomidae (20,809 individuals per m^2) were highly abundant in herbicide-treated sites compared to remnant marsh (3,472 individuals per m^2) and *P. australis* (2,621 individuals per m^2) sites. Caenidae were also associated with herbicide-treated sites that had higher *Schoenoplectus pungens* (common three-square rush [(Vahl) Palla]) cover. Remnant marsh and *P. australis*-invaded sites had similar community composition, and a higher canopy and more standing dead plant biomass because of their abundant emergent vegetation. Psocoptera (booklice), Anthicidae (ant beetle) and Diptera such as Dixidae, Chloropidae, and Cecidomyiidae were associated with remnant marsh or *P. australis* stands with more standing dead biomass on axis 2, while Erotylidae (fungus beetle) were associated with canopy height. On axis 3, there is a separation between remnant marsh and *P. australis* stands that support Lestidae (spread-winged damselfly) and those that support

Pompilidae (spider-wasps), a parasitic wasp whose host taxa is Dolomedes (fishing spiders) (Merritt et al. 2008).

4.3.3 Correlations between aquatic and emerging invertebrates

After excluding taxa that did not have both an aquatic juvenile stage and a winged adult stage there were 17 taxa in the aquatic invertebrate dataset and 31 in the emerging invertebrate dataset (Appendix 3E). Aquatic invertebrates and emerging invertebrates in remnant marsh (Procrustes analysis; $t = 0.865$, $p = 0.049$) and *P. australis* stands (Procrustes analysis; $t = 0.894$, $p = 0.006$) had significant congruence with relatively high t-values, while herbicide-treated habitat had lower, non-significant congruence (Procrustes analysis; $t = 0.485$, $p = 0.647$). NMDS ordinations visualizing aquatic and emerging invertebrate communities by habitat type are presented in Appendix 3F.

4.4 Discussion

Aquatic macroinvertebrate communities and emerging macroinvertebrate communities each showed distinct patterns among the three marsh habitat types. The densities of aquatic and emerging invertebrates were significantly higher in herbicide-treated sites than *P. australis*-invaded or remnant uninvaded marsh habitat. The aquatic invertebrate community composition in *P. australis*, remnant marsh, and herbicide-treated sites were all distinct from one another. In contrast, the emerging invertebrate community composition was similar in *P. australis* and remnant marsh, while herbicide-treated sites were distinct. There was high agreement between the aquatic invertebrate samples and emerging invertebrate samples in *P. australis* and remnant marsh, indicating many taxa present in the aquatic stages emerged at those sites. Correlation between the two communities was low at herbicide-treated sites, suggesting that some

macroinvertebrates with a winged adult stage preferentially emerge elsewhere in the marsh. Our results indicate that *P. australis* supports a similar emerging macroinvertebrate community as remnant uninvaded marsh habitat, at least when examined to the Family-level, and that the macroinvertebrate community in herbicide-treated sites does not resemble either remnant marsh or *P. australis*-invaded marsh two years after treatment.

The taxonomic richness and community evenness of aquatic invertebrates was similar between *P. australis* and remnant uninvaded marsh. Our findings contrast with work from the southern shore of Lake Erie that indicated benthic invertebrate diversity increased with *P. australis* cover, though our results do agree with their finding that densities were similar between *P. australis* and uninvaded marsh (Holomuzki and Klarer 2010). Another study in Lake Erie marshes also found higher densities of aquatic macroinvertebrates in *P. australis* compared to *Typha,* which was a result of gastropod and chironomid abundance (Kulesza et al. 2008). In our study, the total densities for both chironomids and gastropods were similar between *P. australis* and remnant uninvaded marsh. Despite similarities in richness, evenness, and density the aquatic macroinvertebrate community composition was significantly different between *P. australis* and remnant marsh. Numerous aquatic (e.g., Hydrophilidae, Stratiomyidae) and semi-aquatic (e.g., Staphylinidae, Collembola, Dolichopodidae) taxa had their densities strongly, positively associated with remnant marsh whereas none of the taxa densities were strongly positively correlated with *P. australis* sites. The varying water depths and diverse vegetation in remnant marsh may provide more aquatic habitat diversity for macroinvertebrates than the less diverse *P. australis* habitat. Similar patterns were observed in North American brackish marshes, where native vegetation provided more refuge for macroinvertebrates than *P. australis* (Angradi et al. 2001).

While there was a difference in the community composition of aquatic macroinvertebrates between *P. australis* and remnant marsh, the emerging macroinvertebrate community in *P. australis* and remnant uninvaded marsh were similar. *Phragmites australis* and remnant marsh vegetation both supported aquatic, semi-aquatic, and terrestrial taxa including multiple Families of Coleoptera (Anthicidae, Erotylidae, Latridiidae) and Diptera (Dixidae, Cecidomyiidae, Chloropidae). These taxa were associated with the higher canopy height and amount of standing dead biomass characteristic of *P. australis* and remnant marsh habitat. Our assessment of concordance also determined there was high agreement between the aquatic invertebrate community and emerging invertebrate community in both *P. australis* and remnant marsh. We expect this is because of the abundant above-ground biomass in both vegetation communities that provided substrate for emerging macroinvertebrates. Few studies have compared emerging invertebrate communities among *P. australis* and native vegetation, and fewer still have done so in freshwater marshes. In a brackish *Spartina* marsh (New Jersey, USA) Gratton and Denno (2005) found that the macroinvertebrate community in *P. australis* was significantly different than the community in *Spartina,* and was characterized by Collembola, chironomids, and a reduction in marsh spiders due to the structural changes of invasion (Gratton and Denno 2005). In the wetlands of the Great Salt Lake (Utah, USA), *P. australis* provided adequate habitat for both aquatic and terrestrial arthropods and supported similar assemblages as native hardstem bulrush (*Schoenoplectus acutus*) and alkali bulrush (*Bolboshoenus maritimus*) habitat (Leonard et al. 2021). However, native pickleweed (*Salicornia rubra*) had a significantly different arthropod assemblage than the other three vegetation types which is attributed to this vegetation community having less water, litter, and vegetation biomass (Leonard et al. 2021). The similarities in macroinvertebrate communities between *P. australis* and remnant marsh in

our study also indicate that habitat characteristics, such as plant biomass and litter, are important determinants of the emerging macroinvertebrate communities. As presented in Leonard et al. (2021), some native vegetation may support unique macroinvertebrate communities which, if replaced by *P. australis,* will lead to a loss of diversity.

Herbicide-treated sites had significantly different aquatic and emerging invertebrate communities that were characterized by high densities, low taxonomic richness, and unique community composition. Both aquatic and emerging invertebrate samples from herbicide-treated sites were dominated by Diptera, particularly Chironomidae. The herbicide-based control of *P. australis* caused an abrupt change in environmental conditions due to mass macrophyte die-offs. One- to two- years after treatment, macrophyte re-growth in treated sites consists primarily of submerged or floating aquatic vegetation and sparse emergent plant cover, such as cattail or rushes (Robichaud and Rooney 2021a). This large die-off of macrophytes can lead to environmental characteristics that favour Chironomidae. Chironomids are fast-growing and opportunistic, and experiments have demonstrated they will preferentially select sites with high food quality in organically enriched ecosystems (de Haas et al. 2006). In Long Point, herbicide treatment resulted in significantly more submerged litter than in either *P. australis* or uninvaded marsh (Yuckin 2018), which likely provided habitat and high-quality food sources for these detritivores. The high densities of Chironomidae we observed also align with the results from studies specifically examining the respond of invertebrates to glyphosate. After a glyphosate-based herbicide was directly applied to wetlands chironomid abundances increased significantly following macrophyte die-offs (Baker et al. 2014). In the Prairie Pothole Region, the removal of *Typha* spp. using a glyphosate-based herbicide also resulted in higher abundances of Corixidae and Chironomidae (Linz et al. 1999). Following mechanical dredging to increase interspersion

in *P. australis* stands in Long Point, there was no difference in aquatic macroinvertebrate richness or diversity between natural ponds and dredged ponds, but there were higher abundances of macroinvertebrates in dredged ponds because of high Chironomidae abundance (Schummer et al. 2012). As a disturbance-tolerant taxon, chironomid appear to response positively to wetland management that results in the disturbance of plant biomass whether via chemical or mechanical means.

In addition to a unique community composition, the concordance between aquatic and emerging invertebrate communities was low and non-significant in herbicide-treated sites. We attribute this discrepancy in treated marsh to the lack of emergent vegetation, which likely limited the ability of certain taxa to emerge and selects against taxa, such as Cecidomyiidae, that require vegetation for a portion of their life cycle. Some herbicide-treated sites were characterized by higher densities of Caenidae (Ephemeroptera). These same sites had higher abundances of emergent vegetation (i.e., *Schoenoplectus pungens*), which likely served the important role of providing a substrate that aquatic nymphs can use to crawl out of the water and molt. In herbicide-treated sites with less vegetation, the sparse emergent canopy led to warmer water temperatures as more direct sunlight reached the surface of the water and high densities of chironomids were positively associated with these sites. As chironomids can float to the surface of the water and emerge, they are not limited by a lack of emergent vegetation or substrate the way other invertebrate taxa are. In general, warm water temperatures and high-quality food correlates with shorter life cycles in chironomids (Merritt et al. 2008) which may explain why we saw such high densities emerging in these sites. The sparse emergent vegetation and large amount of decomposing biomass resulting from *P. australis* control favours Chironomidae over taxa that emerge by crawling up vegetation or onto land.

While it can be challenging to identify clear relationships between macroinvertebrates and wetland vegetation communities (Batzer 2013), our research highlights the importance of looking at different components of the broader invertebrate community. Incorporating multiple microhabitats (e.g., standing vegetation, submersed vegetation, litter, water column) can provide a more holistic assessment of how macroinvertebrates use wetland habitat and how macroinvertebrates may respond to invasive plant species or invasive species control actions (Harvey et al. 2014). It is also likely that many of the differences among studies are a result of differing sampling approaches and the use of univariate measurements (e.g., diversity indices) that do not capture changes in community composition. In our own study, *P. australis* and remnant marsh had similar emerging invertebrate communities but there were significant differences in the aquatic invertebrate communities they supported. If sampling had been restricted to only submerged vegetation or emergent vegetation, we may have missed important ecological linkages, such as the lack of emergent vegetation influencing emerging invertebrate communities in herbicide-treated sites or that *P. australis* supported significantly different aquatic macroinvertebrate communities. Similarly, comparing coarse measurements of macroinvertebrate communities using alpha diversity or evenness can mask important changes in trophic structure (e.g., Gratton and Denno 2005) or changes in taxa identity.

Assessing the response of native biota to invasive species control is essential for measuring the success of wetland restoration projects. Comparing the way native biota use invaded, uninvaded, and treated habitat can inform land management by weighing the consequences of unmitigated invasion against potential unintended impacts of invasive species control. In the case of *P. australis*, the effects of invasion include altering the aquatic macroinvertebrate community composition, but our results also emphasize that efforts to control

invasive plant species can have significant effects on the ecosystem that last well beyond the initial treatment. The return of native vegetation following *P. australis* removal can lead to the recovery of macroinvertebrate communities (e.g., Gratton and Denno 2005) but, in freshwater coastal marsh systems such as Long Point, it may take longer for native vegetation to recover to pre-treatment abundance (e.g., Robichaud and Rooney 2021a). Measuring changes in the macroinvertebrate community is important as macroinvertebrates can be indicators of ecological condition and are also key components of aquatic food webs and aquatic-terrestrial linkages (e.g., Collier et al. 2002). We highly recommend that future work includes sampling both the aquatic and emerging macroinvertebrate communities in these marshes to assess recovery. Given the importance of a diverse macroinvertebrate community, a successful restoration trajectory should result in eventual convergence with the remnant, uninvaded marsh macroinvertebrate community.

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5 General Conclusion

5.1 Thesis Overview

5.1.1 Synthesis

The research presented in this thesis represents the comprehensive biological monitoring of wetland communities after the control of an established invasive species (*Phragmites australis*) using a glyphosate-based herbicide. At the individual level, we first assessed what mechanisms contribute to the success of *P. australis* at the expense of resident plant species (Chapter 2). Then, we scaled up and assessed the efficacy of herbicide-based control of *P. australis* and how the plant communities recovered when allowed to passively recolonize treated areas (Chapter 3). Finally, we assessed the macroinvertebrates community in *P. australis* stands and herbicide-treated sites and compared them to remnant, uninvaded marsh (Chapter 4). A conceptual model linking each chapter is presented in Figure 5.1.

5.1.2 Chapter 2: Competition for light resources and niche space overlap between P. australis *and resident species.*

Identifying the mechanisms that lead to the displacement of resident species by a non-native species can be difficult. By applying coexistence theory (Chesson 2000) to invasive plant species, MacDougall et al. (2009) suggested that the ability of an invasive species to establish and create a negative impact can be better predicted by niche differences (e.g., traits that are self-limiting when the species is abundant, such as deeper rooting depth) and fitness differences (e.g., traits that are density independent and favour one competitor over another such as faster nutrient uptake) than niche overlap alone. Specifically, detrimental invasive species will take up shared limited resources more efficiently than their neighbour resident species. While there is clear evidence that invasive *P. australis* is successfully displacing resident species, there remains few

field studies that attempt to quantify the niche overlap and fitness difference between *P. australis* and the species it is displacing.

To quantify niche and fitness difference between *P. australis* and resident wetland plants, we measured performance of resident wetland species *Calamagrostis canadensis, Carex aquatilis,* and *Typha* growing with or without above-ground competition from *P. australis.* Our results indicated that *P. australis* can intercept more photosynthetically active radiation than resident species and is able to assimilate more carbon, more efficiently over the growing season. All resident species had lower carbon assimilation rates when growing in competition with *P. australis,* including *Typha,* which produced a similar amount of above-ground biomass as *P. australis*. In contrast, *P. australis* did not experience negative effects from competition with resident species and assimilated similar amounts of carbon with or without inter-specific competition. *Phragmites australis* also had the largest niche space of all four species, reflecting its wide range of environmental tolerance – in particular, soil moisture and nutrient levels - that have assisted its establishment throughout North America. Resident species had a high probability of overlap onto the niche space of *P. australis,* indicating it is excluding resident species from areas they would otherwise be established. These results provide evidence that the ability of *P. australis* to reduce the availability of a required resource (PAR) and more efficiently use it over the growing season, while exhibiting high niche overlap with resident species, likely contributes directly to its success in North American freshwater wetlands.

5.1.3 Chapter 3: Efficacy of glyphosate-based herbicide and recovery of native vegetation follow P. australis *control.*

The documented negative effects of invasive *P. australis* prompted the Ontario Ministry of Natural Resources and Forestry, the Nature Conservancy of Canada, and other partners to

begin a large-scale *P. australis* control project in Long Point peninsula and Rondeau Provincial Park. The application of a glyphosate-based herbicide directly over standing water is new in Canada and the removal of approximately 400 ha of a well-established invasive species may have unintended consequences for native biota. Thus, this *P. australis* control project in two ecologically significant marshes warrants a comprehensive monitoring of the biological communities. In this chapter, we evaluate how effective herbicide application was at reducing *P. australis* populations and what native vegetation returned two years after treatment.

We found that one year after treatment, herbicide suppressed over 99% of *P. australis* and worked effectively along the entire water depth gradient (10 – 48 cm). Re-growth of *P. australis* was minimal but did occur, particularly in the second year after treatment. This re-growth emphasizes the requirement of long-term maintenance to keep *P. australis* population levels low after treatment. Additionally, high water levels in Lake Erie resulted in flooding in both marshes in the two years following treatment which influenced the trajectory of the native vegetation. This flooding likely aided the *P. australis* suppression, however, by preventing seedlings from germinating. After treatment, some sites resembled emergent marsh reference conditions but approximately half of the sites were characterized by abundant cover of non-native *Hydrocharis morsus-ranae* (European frogbit).

5.1.4 Chapter 4: A comparison of the macroinvertebrates in herbicide-treated sites, P. australis *stands, and uninvaded remnant marsh.*

Macroinvertebrates are a critical component of wetland ecosystems. Macroinvertebrates provide essential food for birds, herptiles, and other invertebrates while emerging taxa are a key link between aquatic and terrestrial ecosystems. Despite their importance, the response of wetland macroinvertebrates to *P. australis* invasion and control actions in freshwater marshes are

not well understood. Further, much of the available research on the subject focuses on benthic invertebrates which neglects the other microhabitats that macroinvertebrates can use (e.g., Harvey et al. 2014). To better understand how invasive *P. australis* and *P. australis* control actions affect macroinvertebrate communities, we assessed both the aquatic invertebrate community (e.g., submersed vegetation samples) and emerging invertebrate community (e.g., emergence trap samples) in *P. australis* stands, herbicide-treated sites, and remnant marsh.

Herbicide-treated sites, which were sampled two years after herbicide treatment, had significantly higher densities of invertebrates than either remnant marsh or *P. australis* stands. However, these sites were dominated by tolerant generalist taxa, particularly Chironomidae and Oligochaeta. In contrast, the abundance, richness, and evenness of macroinvertebrates present in *P. australis* stands were similar to those present in uninvaded remnant marsh, and the emerging macroinvertebrate communities were indistinguishable. This suggests that, in these freshwater coastal wetlands, the presence of *P. australis* is not detrimental to macroinvertebrate communities assessed at the Family level. The change in macroinvertebrate community composition after herbicide-treatment could benefit certain wetland taxa, such as aerial insectivore birds which consume flying invertebrates on the wing, but the reduction in diversity may mean a loss of functional traits in treated sites (e.g., dominance by detritivores).

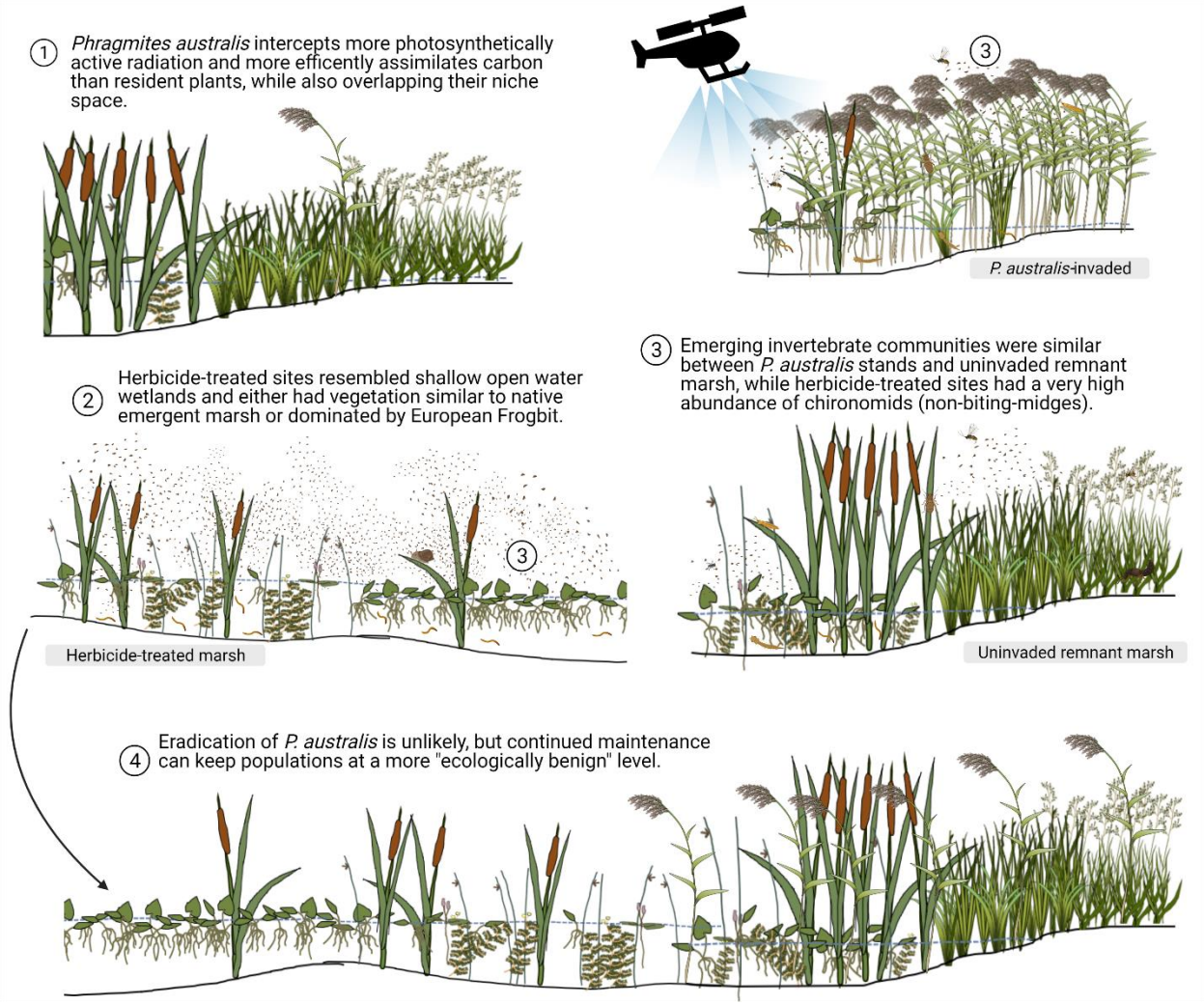

Figure 5.1. Conceptual diagram of key findings regarding *P. australis* establishment and competition, and the composition of plant and macroinvertebrate communities after *P. australis* control. Image was created with BioRender and The Noun Project.

5.2 Emergent properties

The research presented in this thesis, when considered as a whole, can help inform wetland restoration approaches when contending with invasive species and provides insight into

the response of wetland communities to large-scale invasive species control in freshwater marshes.

5.2.1 Invasive *Phragmites australis* management implications

The non-selective glyphosate-based herbicide used in Long Point and Rondeau was extremely effective at reducing populations of *P. australis* across a range of standing water depths. However, in the second year after treatment, *P. australis* has already begun to return to numerous sites. The ability of *P. australis* to recover after control action is well documented (e.g., Martin and Blossey 2013), and it can be particularly challenging to eradicate when trying to control large stands (Quirion et al. 2017). As such, continued maintenance is a required part of *P. australis* management plans. The choice of tool used for this maintenance should be carefully considered against the potential for harm. Within our study system, the concentrations of glyphosate we detected did not approach thresholds of toxicological concern and dissipated rapidly from water (within 30 days) (Robichaud and Rooney 2021). Given the high tolerance of invertebrates to glyphosate, we do not consider the differences in macroinvertebrate community composition we detected two-years post treatment to be attributed to residual glyphosate toxicity. For example, the reproductive no-observed-effects-concentration for earthworms (*Eisenia fetida*), springtails (*Folsomia candida*), and predatory mites (*Hyoaspis aculeifer*) was 472.8 mg glyphosate acid equivalent/kg dry soil (von Mérey et al. 2016), and in an assessment of glyphosate toxicity on aquatic invertebrates, the most sensitive species was *Daphnia magna* which had a 48-hour EC_{50} of 9.7 mg Round Up/L (Giesy et al. 2000). In Long Point and Rondeau, the highest concentration of glyphosate in water was 0.320 mg/L and 0.250 mg/Kg in sediment, well below these thresholds (Robichaud and Rooney 2021).

It is worth noting, however, that low concentrations of glyphosate residue remained detectable in sediment for up to one year after treatment (Robichaud and Rooney 2021). These findings indicate that, if follow-up applications of a glyphosate-based herbicide occur repeatedly and regularly in the same vicinity, there is the potential for herbicide residue to accumulate in sediments. Other research has also noted the potential for repeated applications to lead to the accumulation of herbicide residue (Myers et al. 2016, Sesin et al. 2020) or even present a risk to wetland food webs by accumulating in attached algal communities (e.g., Beecraft and Rooney 2021). Therefore, we caution the use of herbicide application as a default long-term treatment approach. A diverse toolkit, including biocontrol (Blossey and Casagrande 2016), mechanical removal (Hazelton et al. 2014), or active re-seeding (see section 5.2.2.) may limit *P. australis* abundance without the use of herbicide. In cases where herbicide is used, we recommend concurrent ecosystem monitoring to assess the concentration of herbicide, and associated breakdown products, in the environment and the risk to non-target species.

5.2.2 Restoration and re-seeding

Removing an invasive species creates a vacant niche that must be filled, but it does not correct the underlying environmental conditions that resulted in invasion in the first place. In Lake Erie, this includes agriculture and urban development in the surrounding landscape (e.g., Herdendorf 1992), changes to the shoreline (e.g., Gottgens et al. 1999), high nutrient loading (e.g., King et al. 2007), the presence of multiple invasive species (e.g., Trebitz and Taylor 2007), and the stress of climate change (Hayhoe et al. 2010). Surrounding land use and nutrient pollution drive the dominance of *P. australis* and other invasive species, like *Typha angustifolia*, in wetlands. Invasive species, including *P. australis,* were more likely to be dominant in Great

Lakes wetlands that had high agricultural intensity in the surrounding watershed (Trebitz and Taylor 2007). Simulations of Great Lakes wetlands have determined that the invasion success of *P. australis* is linked to nitrogen accumulation (Sharp et al. 2021) and exhibits a threshold response: at low nitrogen loading rates (< 5 g N m^{-2} yr^{-1}) *P. australis* coexisted with the native plant community but at high loading rates (≥ 15 g N m^{-2} yr^{-1}) it was almost or completely dominant (Martina et al. 2016). In nitrogen-rich environments, *P. australis* produces more biomass and is more likely to displace resident plant species (Rickey and Anderson 2004). Given results from Chapter 2 that *P. australis* is more efficient at using shared resources than native graminoids, and that *P. australis* thrives in disturbed areas (e.g., Minchinton and Bertness 2003), it is likely *P. australis* will rapidly recolonize treated areas left to passive restoration.

However, even if *P. australis* was completely eradicated from the region, there are numerous other invasive species that may take its place. Secondary invasion is often the result of targeted invasive species control (Pearson et al. 2017), and the conditions of Lake Erie make this a likely outcome. A 2007 survey of the Great Lakes determined that exotic or invasive strains of plants were prevalent in the wetlands of Lake Erie (Trebitz and Taylor 2007). These included *Myriophyllum spicatum* (Eurasian water-milfoil [L,]), *Najas minor* (brittle water nymph [All.]), *Potamogeton crispus* (curly leaved pondweed [L.]), *Butomus umbellatus* (flowering rush [L.]), *Lythrum salicaria* (purple loosestrife [L.]), *Phalaris arundinacea* (reed canary grass [L.]), *Phragmites australis* and *Typha angustifolia* and its hybrid *T.* x *glauca* (Trebitz and Taylor 2007). At the time this study was published, *Hydrocharis morsus-ranae* was not yet present in Lake Erie, though this has since changed. Based on results from Chapter 3, other invasive species (primarily *Hydrocharis morsus-ranae*) have benefited from *P. australis* control in Long Point. The likely outcome of such 'passive restoration,' where the invasive plant is removed but

the plant community is then left to recover without assistance (i.e., Morrison and Lindell 2010), is a plant community characterized by new invasive species (secondary invasion) or the eventual re-establishment of *P. australis.* Active planting or seeding native species, however, can assist native vegetation and rapidly fill the open niche space left by *P. australis* removal.

Facilitating or planting native vegetation can pre-empt invasive species by reducing the availability of resources (Hussner et al. 2017). However, as our findings demonstrate in Chapter 2, overlap in niche space with commonly dominant resident graminoids is not enough to repel *P. australis* in freshwater marshes. *Phragmites australis* had a very high water use efficiency and its average carbon assimilation rate (19.6 µmol CO_2 s^{-1} m^{-2} (± 0.51 st. error) at 1500 µmol $s^{-1}m^{-2}$) was higher than *Typha,* which is another problematic wetland invader (Bansal et al. 2019). This advantage in carbon assimilation, coupled with the detrimental effect that competition with *P. australis* has on resident species, explains a component of the success of *P. australis* in freshwater marshes. Large fitness differences (Chesson 2000) drive invasion impact – if an invasive species is more effective at taking up resources required by resident plants it will result in the eventual dominance of that species (MacDougall et al. 2009). Seeding a wetland to establish a native community while also preventing re-invasion takes a careful consideration (see Kettenring and Tarsa 2020). Once an appropriate seed mixture is determined, early seeding is an option that can keep invasion under control. Native seeds sown early in the season can establish before invasive species that are also in the seedbank, reducing the population of the invasive species and allowing for quick and effective follow-up treatments to kill the unwanted invader (Kettenring and Tarsa 2020). This approach balances active follow-up, recognizing the invasive species may never be fully eradicated, with the establishment of a diverse native community.

Active planting of emergent vegetation may also benefit the macroinvertebrate community by encouraging a more rapid return to the dense vegetation that many taxa rely on.

5.2.3 Biological Monitoring

The need for comprehensive monitoring to assess the trajectory of a restored ecosystem is a common refrain, for good reason. Ecosystems can change slowly, and long-term monitoring captures these changes, which can then inform restoration practices and provide an important basis for future experimentation (Lovett et al. 2007). Wetland restoration with the intent of recovering lost biodiversity is challenging (Zedler and Kercher 2005), and the structure and function of restored wetlands rarely recover to pre-degradation levels, even when it has been nearly a century since restoration took place (Moreno-Mateos et al. 2012). Monitoring key ecological variables is required to determine the success of restoration and inform appropriate adaptive management approaches (Thom 2000).

In Long Point and Rondeau, the dramatic disturbance of removing hundreds of hectares of dense *P. australis,* coupled with high water levels, provided an opportunity for a unique vegetation community to assemble, which included a high abundance of non-native *Hydrocharis morsus-ranae*. Non-native species and a lack of native propagules are common reasons wetland restoration does not meet intended goals (e.g., Matthews and Spyreas 2010). As identified in the previous section, active re-seeding may be able to prevent the dominance of non-native species, but it requires a thorough understanding of the ecosystem. Long-term monitoring of a site is necessary to evaluate the success of passive or active restoration and can provide evidence of legacy effects that affect successful revegetation (Kettenring and Tarsa 2020). In a 2014 review, 71% of *P. australis* control projects conducted monitoring for only one to three years after

treatment and focused on only *P. australis* or vegetation metrics (Hazelton et al. 2014). The drastic change in vegetation cover after *P. australis* control led to an equally drastic change in macroinvertebrate communities – from the diverse emerging invertebrate community present in *P. australis* to the less diverse, Chironomidae-dominated community we observed in herbicide-treated sites. While this may provide more prey items for wetland biota, it also indicates that managers should ensure some dense emergent vegetation (preferably remnant resident vegetation) remains so that macroinvertebrates have a refuge after invasive species control. Long-term monitoring of macroinvertebrate communities would provide important data regarding if, and when, macroinvertebrate communities in herbicide-treated sites begin to resemble those in uninvaded sites. The response of both the vegetation and macroinvertebrate communities is influenced both by invasive *P. australis* removal and prevailing high Lake Erie water levels. As coastal marshes are dynamic systems, we expect that sometime within the next decade water levels will drop, providing an opportunity for new seeds to germinate and affecting macroinvertebrate communities. Lower water levels will also favour *P. australis,* and thus a robust monitoring program would provide important early warnings about re-invasion, secondary invasions, and the recovery of native species.

5.3 Future directions

This thesis represents a comprehensive ecological assessment of the response of native biota to invasive *P. australis* and to large scale, herbicide-based control of *P. australis* in ecologically significant wetlands. By integrating concepts from coexistence theory, community ecology, invasion biology and ecological restoration my work provides insight into the mechanisms that assist *P. australis* dominance, and the response of vegetation and

macroinvertebrate communities. Importantly, there are numerous additional avenues and new research questions generated by this body of work.

5.3.1 *Testing coexistence theory more directly with* P. australis

The interspecific competition work that we conducted with *P. australis* represent a starting point for assessing the roles of niche differences and fitness differences in the success of *P. australis* invasions. However, our work is missing key elements necessary to be a true test of fitness and niche differences or stable coexistence. We suggest that future work incorporates a measure of *intra*specific competition for each species to assess niche differences more effectively. This could include a measurement of self-shading vs shading of resident plants, nutrient competition via rooting depth, or similar density-dependent factors that may provide *P. australis* an opportunity to establish in a resident community. While we have identified more efficient photosynthetic performance as a density-independent factor that contributes to *P. australis* dominances, there are many other potential fitness differences that could benefit *P. australis* including novel weapons (i.e., allelopathy (Uddin and Robinson 2017)), release from enemies (e.g., Allen et al. 2015), or differences in nutrient uptake (e.g., Mozdzer et al. 2010). Considering the vast literature on invasive *P. australis* it is possible that a first step in teasing apart the niche and fitness differences that lead to its high impact as an invader would be a systematic review or meta-analysis. In an experimental approach, this work would benefit from multi-year measurements of population level changes as coexistence is influenced by temporal and spatial differences in resources (e.g., Chesson and Huntly 1997). Researchers have conducted elegant and successful stand-level competition experiments using *P. australis* and *Typha* sp. (Paradis et al. 2014) that could be adapted to address this research need.

5.3.2 Long-term monitoring and experimental water level manipulations

Continued comparisons of the vegetation and macroinvertebrate communities among herbicide-treated, remnant uninvaded marsh and *P. australis* stands would provide valuable insight into the recovery of native communities. Additionally, *H. morsus-ranae* is now abundant in nearly half of the treated sites. It would be a worthwhile endeavor to evaluate how wetland biota use sites that are heavily dominated by *H. morsus-ranae* compared to those that consist of native submersed and floating vegetation. Unlike *P. australis*, performing manipulative experiments with *H. morsus-ranae* would be feasible as it is a floating plant – for example, establishing plots with varying densities of *H. morsus-ranae* could provide insight into how wetland fauna (macroinvertebrates, fish, herptiles, or birds) use differing habitat. This would provide valuable evidence for managers trying to make the decision of whether to control *P. australis* when secondary invasion from *H. morsus-ranae* is likely.

Flooding is one of the main drivers that influences wetland communities. High water levels have selected for submerged, floating, and robust emergent (e.g., cattail) species in treated areas of Long Point and Rondeau. Experimentally manipulating water depths, whether in a greenhouse or in the field, would yield important data on what species could emerge when water levels drop. This would provide insight into how feasible passive restoration is in treated marshes and identify if re-seeding is appropriate to encourage the establishment of native vegetation. Water level declines have historically resulted in *P. australis* germinating from invaded seedbanks (e.g., Wilcox 2012), thus evaluating which species are present in seed banks before water levels decline in the field could give practitioners an opportunity to plan management accordingly. High water levels may also affect macroinvertebrate communities in coastal marshes. *Phragmites australis* control actions, coupled with high water levels, resulted in

areas of the marsh that were consistently flooded. This shallow open water habitat is likely ideal fish habitat which may influence the emerging invertebrate community composition. For example, fish predation could contribute to the lower taxonomic richness if certain taxa were preferentially consumed (Batzer and Wissinger 1996). Future research identifying the fish community using these wetlands would be beneficial to identify the ecological linkages between *P. australis* removal, changes in wetland habitat, and native biotic communities.

Finally, this work constituted a relatively thorough evaluation of the macroinvertebrate communities present in vegetation communities of interest, but macroinvertebrate identification was only completed to the lowest feasible taxonomic level. Taking identifications past Family, to Genus or even Species, could yield further insight into differences in emergence timing, different microhabitat use, or more nuanced responses to invasion. As traditional microscope identification may not be feasible for the tens of thousands of invertebrates these sampling methods yield, a DNA metabarcoding approach (e.g., Beermann et al. 2018) is possible to strike a balance between taxonomic resolution and effort.

5.4 Concluding remarks

Restoration is critically important for a planet that has lost so many of its natural wetlands. My work demonstrates that controlling established populations of *P. australis* in coastal freshwater marshes is possible, but that there are multiple underlying factors that make recovery of native communities challenging. Passive restoration is likely to lead to reinvasion or secondary invasions in Lake Erie coastal wetlands but integrating a better understanding of competition mechanisms can inform the active restoration of plant communities that will better resist invasion. Finally, my work also demonstrates that aquatic macroinvertebrates respond to

both *P. australis* invasion and *P. australis* herbicide-based control. These results will contribute to improving the ability of land managers in making decisions about *P. australis* control in freshwater marshes.

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Appendices

Appendix 1A. Location of the 96 clip plot pairs in Long Point, ON established in 2017. Location of 2016 plots was nearly identical to the 2017 layout. Inset maps illustrate the location of the phytometers in Long Point, with a polygon indicating the area from which the environmental measures were taken from unmanipulated areas of each target species, and the location of the study in the Great Lakes region.

Appendix 1B. Phytometer height over the 2016 field season. Not collected (NC) indicates when a phytometer had died or was consumed. If above-ground tissue was still present it was measured and collected at the end of the growing season, though occasionally phytometers did not possess a whole, entire leaf for carbon assimilation measurements.

ID	Phytometer	Neighbours	Competition	18-May-16	03-Jun-16	12-Jun-16	22-Jun-16	02-Jul-16	12-Jul-16
PCC1CU	*Phragmites*	*Calamagrostis*	No	63	160	183	216	230	248
PCC2CU	*Phragmites*	*Calamagrostis*	No	41	98	110	126	129	145
PCC1	*Phragmites*	*Calamagrostis*	Yes	60	128	155	173	183	188
PCC2	*Phragmites*	*Calamagrostis*	Yes	42	100	124	141	149	160
PCC3CU	*Phragmites*	*Calamagrostis*	No	52	127	145	166	180	194
CAP1CU	*Carex*	*Phragmites*	No	61	92	91	100	101	98
CAP1	*Carex*	*Phragmites*	Yes	61	92	97	97	97	97
CAP2CU	*Carex*	*Phragmites*	No	71	80	86	91	89	90
CAP2	*Carex*	*Phragmites*	Yes	70	91	93	95	93	93
CAP3CU	*Carex*	*Phragmites*	No	70	106	107	103	112	110
CAP3	*Carex*	*Phragmites*	Yes	70	99	105	105	104	103
CAP4CU	*Carex*	*Phragmites*	No	64	105	114	111	111	NC
CAP4	*Carex*	*Phragmites*	Yes	61	105	112	113	113	114
CAP5CU	*Carex*	*Phragmites*	No	83	90	94	94	95	90
CAP5	*Carex*	*Phragmites*	Yes	82	100	104	106	107	100
PCC3	*Phragmites*	*Calamagrostis*	Yes	52	138	153	167	174	192
PCC4CU	*Phragmites*	*Calamagrostis*	No	75	190	222	243	261	279
PCC4	*Phragmites*	*Calamagrostis*	Yes	78	191	214	245	258	272
PCC5CU	*Phragmites*	*Calamagrostis*	No	63	174	202	230	249	264
PCC5	*Phragmites*	*Calamagrostis*	Yes	64	160	185	212	231	243
PCC6CU	*Phragmites*	*Calamagrostis*	No	69	122	132	146	156	166
PCC6	*Phragmites*	*Calamagrostis*	Yes	65	159	178	209	220	223
CCP1CU	*Calamagrositis*	*Phragmites*	No	66	106	114	120	123	124
CCP1	*Calamagrositis*	*Phragmites*	Yes	67	93	98	109	114	114
CCP2CU	*Calamagrositis*	*Phragmites*	No	65	83	90	NC	NC	NC
CCP2	*Calamagrositis*	*Phragmites*	Yes	79	113	119	131	134	137

ID	Phytometer	Neighbours	Competition	18-May-16	03-Jun-16	12-Jun-16	22-Jun-16	02-Jul-16	12-Jul-16
CCP3CU	*Calamagrositis*	*Phragmites*	No	67	108	129	139	NC	NC
CCP3	*Calamagrositis*	*Phragmites*	Yes	61	89	97	101	101	103
CCP4CU	*Calamagrositis*	*Phragmites*	No	58	77	82	85	79	NC
CAP6CU	*Carex*	*Phragmites*	No	59	85	96	108	108	112
CAP6	*Carex*	*Phragmites*	Yes	63	105	100	100	101	100
CAP7CU	*Carex*	*Phragmites*	No	62	78	102	111	106	109
CAP7	*Carex*	*Phragmites*	Yes	63	103	114	120	119	119
CAP8CU	*Carex*	*Phragmites*	No	53	83	82	89	85	84
CAP8	*Carex*	*Phragmites*	Yes	51	81	86	86	86	86
CAP9CU	*Carex*	*Phragmites*	No	71	96	98	99	100	102
CAP9	*Carex*	*Phragmites*	Yes	73	109	110	113	111	111
CAP10CU	*Carex*	*Phragmites*	No	80	88	100	104	100	105
CAP10	*Carex*	*Phragmites*	Yes	79	93	99	99	100	100
PCA1CU	*Phragmites*	*Carex*	No	86	149	171	194	208	223
PCA1	*Phragmites*	*Carex*	Yes	85	116	130	142	154	164
PCA2CU	*Phragmites*	*Carex*	No	80	122	131	148	NC	NC
PCA2	*Phragmites*	*Carex*	Yes	80	169	197	227	246	255
PCA3CU	*Phragmites*	*Carex*	No	69	140	155	175	194	215
PCA3	*Phragmites*	*Carex*	Yes	68	109	123	137	145	160
PCA4CU	*Phragmites*	*Carex*	No	83	130	141	154	176	191
PCA4	*Phragmites*	*Carex*	Yes	85	133	148	164	175	182
PCA5CU	*Phragmites*	*Carex*	No	83	108	NC	NC	NC	NC
PCA5	*Phragmites*	*Carex*	Yes	84	150	163	179	188	201
PCA6CU	*Phragmites*	*Carex*	No	81	120	133	143	148	161
PCA6	*Phragmites*	*Carex*	Yes	83	146	159	175	189	197
CAP11CU	*Carex*	*Phragmites*	No	87	91	97	97	97	97
CAP11	*Carex*	*Phragmites*	Yes	84	88	91	92	92	89
CAP12CU	*Carex*	*Phragmites*	No	88	88	98	98	103	103
CAP12	*Carex*	*Phragmites*	Yes	89	103	108	97	95	108
CCP4	*Calamagrositis*	*Phragmites*	Yes	86	116	141	174	190	191

ID	Phytometer	Neighbours	Competition	18-May-16	03-Jun-16	12-Jun-16	22-Jun-16	02-Jul-16	12-Jul-16
CCP5CU	*Calamagrositis*	*Phragmites*	No	82	109	139	137	NC	NC
CCP5	*Calamagrositis*	*Phragmites*	Yes	83	121	133	161	169	171
CCP6CU	*Calamagrositis*	*Phragmites*	No	55	78	88	94	100	101
CCP6	*Calamagrositis*	*Phragmites*	Yes	57	78	83	89	94	97
CCP7CU	*Calamagrositis*	*Phragmites*	No	55	61	68	74	78	80
CCP7	*Calamagrositis*	*Phragmites*	Yes	56	79	78	80	82	83
CCP8CU	*Calamagrositis*	*Phragmites*	No	45	75	79	83	85	85
CCP8	*Calamagrositis*	*Phragmites*	Yes	43	74	79	87	90	90
CCP9CU	*Calamagrositis*	*Phragmites*	No	62	89	91	95	100	103
CCP9	*Calamagrositis*	*Phragmites*	Yes	63	78	81	89	94	98
CCP10CU	*Calamagrositis*	*Phragmites*	No	57	92	87	110	110	121
CCP10	*Calamagrositis*	*Phragmites*	Yes	58	78	79	80	81	84
CCP11CU	*Calamagrositis*	*Phragmites*	No	55	64	64	65	66	66
CCP11	*Calamagrositis*	*Phragmites*	Yes	53	54	74	57	59	63
CCP12CU	*Calamagrositis*	*Phragmites*	No	68	94	101	109	110	NC
TYP1CU	*Typha*	*Phragmites*	No	85	152	167	185	194	199
TYP1	*Typha*	*Phragmites*	Yes	86	154	180	215	228	239
TYP2CU	*Typha*	*Phragmites*	No	78	140	174	200	213	217
TYP2	*Typha*	*Phragmites*	Yes	82	168	193	229	232	245
TYP3CU	*Typha*	*Phragmites*	No	88	153	170	188	198	205
TYP3	*Typha*	*Phragmites*	Yes	85	133	133	133	136	137
TYP4CU	*Typha*	*Phragmites*	No	92	170	187	227	227	248
TYP4	*Typha*	*Phragmites*	Yes	94	176	200	221	237	259
TYP5CU	*Typha*	*Phragmites*	No	98	180	197	229	246	258
TYP5	*Typha*	*Phragmites*	Yes	99	194	208	233	250	272
TYP6CU	*Typha*	*Phragmites*	No	98	185	200	211	242	256
TYP6	*Typha*	*Phragmites*	Yes	97	172	194	200	235	270
PTY1CU	*Phragmites*	*Typha*	No	81	147	158	174	196	213
PTY1	*Phragmites*	*Typha*	Yes	83	139	168	186	198	217
PTY2CU	*Phragmites*	*Typha*	No	93	147	155	169	193	203

ID	Phytometer	Neighbours	Competition	18-May-16	03-Jun-16	12-Jun-16	22-Jun-16	02-Jul-16	12-Jul-16
PTY2	*Phragmites*	*Typha*	Yes	92	170	180	211	221	238
PTY3CU	*Phragmites*	*Typha*	No	85	108	124	141	157	NC
PTY3	*Phragmites*	*Typha*	Yes	90	156	157	181	202	223
PTY4CU	*Phragmites*	*Typha*	No	98	181	198	212	231	249
PTY4	*Phragmites*	*Typha*	Yes	95	165	180	196	218	234
PTY5CU	*Phragmites*	*Typha*	No	87	178	198	221	247	263
PTY5	*Phragmites*	*Typha*	Yes	99	181	192	222	243	262
PTY6CU	*Phragmites*	*Typha*	No	112	174	192	213	228	242
PTY6	*Phragmites*	*Typha*	Yes	117	209	228	259	278	298

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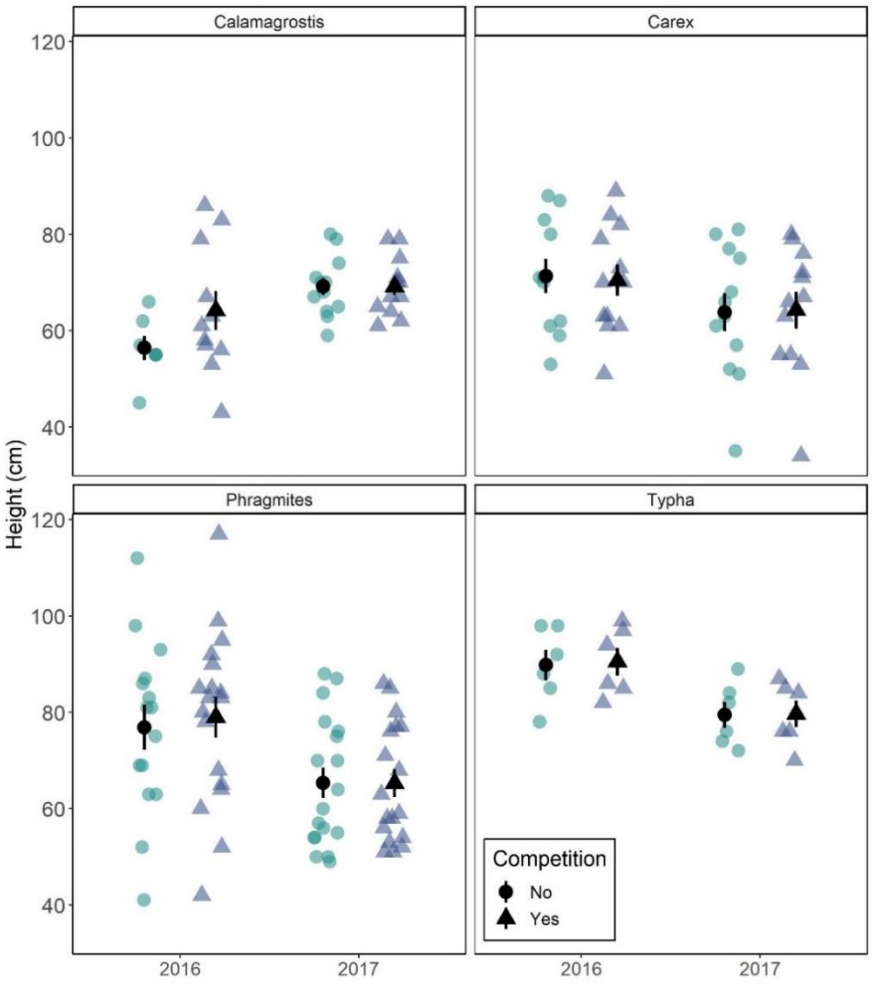
Appendix 1C. Phytometer height over the 2017 field season. Not collected (NC) indicates when a phytometer had died or was consumed. If above-ground tissue was still present it was measured and collected at the end of the growing season, though occasionally phytometers did not possess a whole, entire leaf for carbon assimilation measurements.

ID	Phytometer	Neighbour	Competition	10-May-17	27-May-17	07-Jun-17	16-Jun-17	28-Jun-17
CAP1	Carex	Phragmites	Yes	34	82	75	132	140
CAP10	Carex	Phragmites	Yes	76	117	127	150	210
CAP10CU	Carex	Phragmites	No	77	108	104	142	150
CAP11	Carex	Phragmites	Yes	79	98	120	141	160
CAP11CU	Carex	Phragmites	No	80	100	63	60	61
CAP12	Carex	Phragmites	Yes	71	91	104	103	118
CAP12CU	Carex	Phragmites	No	66	82	91	95	120
CAP1CU	Carex	Phragmites	No	35	75	70	131	130
CAP2	Carex	Phragmites	Yes	55	90	97	116	140
CAP2CU	Carex	Phragmites	No	57	91	96	127	127
CAP3	Carex	Phragmites	Yes	63	105	86	123	125
CAP3CU	Carex	Phragmites	No	61	75	82	115	135
CAP4	Carex	Phragmites	Yes	55	92	81	146	156
CAP4CU	Carex	Phragmites	No	52	70	80	141	143
CAP5	Carex	Phragmites	Yes	72	118	89	144	153
CAP5CU	Carex	Phragmites	No	75	89	90	141	158
CAP6	Carex	Phragmites	Yes	80	114	100	154	163
CAP6CU	Carex	Phragmites	No	81	95	103	142	145
CAP7	Carex	Phragmites	Yes	53	90	104	131	135
CAP7CU	Carex	Phragmites	No	51	71	95	155	140
CAP8	Carex	Phragmites	Yes	66	96	93	134	138
CAP8CU	Carex	Phragmites	No	63	76	80	130	131
CAP9	Carex	Phragmites	Yes	67	108	103	140	160
CAP9CU	Carex	Phragmites	No	68	92	92	143	150
CCP1	Calamagrostis	Phragmites	Yes	61	79	80	88	88
CCP10	Calamagrostis	Phragmites	Yes	70	83	76	85	88

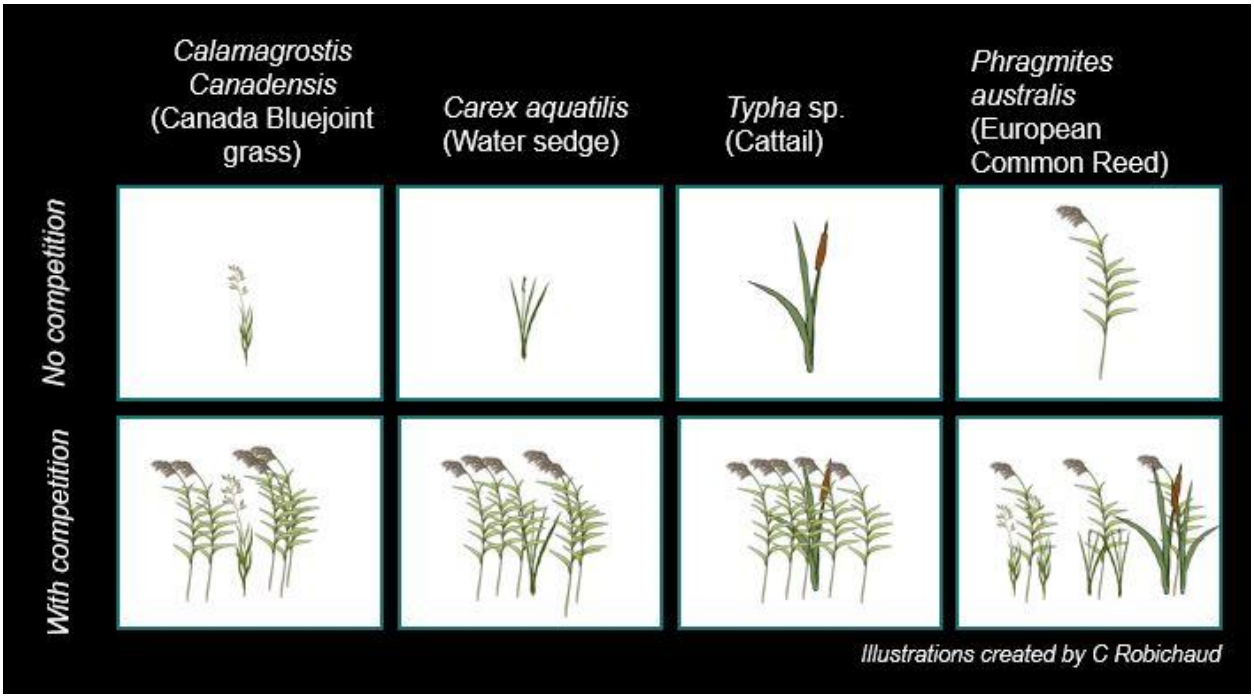
ID	Phytometer	Neighbour	Competition	10-May-17	27-May-17	07-Jun-17	16-Jun-17	28-Jun-17
CCP10CU	Calamagrostis	Phragmites	No	70	85	93	101	110
CCP11	Calamagrostis	Phragmites	Yes	79	97	106	112	120
CCP11CU	Calamagrostis	Phragmites	No	80	100	104	111	111
CCP12	Calamagrostis	Phragmites	Yes	75	101	115	118	118
CCP12CU	Calamagrostis	Phragmites	No	74	103	118	121	125
CCP1CU	Calamagrostis	Phragmites	No	63	67	74	93	100
CCP2	Calamagrostis	Phragmites	Yes	67	81	93	110	145
CCP2CU	Calamagrostis	Phragmites	No	67	87	95	98	110
CCP3	Calamagrostis	Phragmites	Yes	67	95	105	95	95
CCP3CU	Calamagrostis	Phragmites	No	68	82	87	94	96
CCP4	Calamagrostis	Phragmites	Yes	70	90	99	110	118
CCP4CU	Calamagrostis	Phragmites	No	71	93	91	202	115
CCP5	Calamagrostis	Phragmites	Yes	71	99	110	120	124
CCP5CU	Calamagrostis	Phragmites	No	70	90	89	94	95
CCP6	Calamagrostis	Phragmites	Yes	65	104	74	98	100
CCP6CU	Calamagrostis	Phragmites	No	64	86	91	99	105
CCP7	Calamagrostis	Phragmites	Yes	64	84	91	105	112
CCP7CU	Calamagrostis	Phragmites	No	65	86	94	109	110
CCP8	Calamagrostis	Phragmites	Yes	62	86	96	90	90
CCP8CU	Calamagrostis	Phragmites	No	59	96	107	124	130
CCP9	Calamagrostis	Phragmites	Yes	79	107	112	118	135
CCP9CU	Calamagrostis	Phragmites	No	79	81	94	100	103
PCA1	Phragmites	Carex	Yes	56	136	158	193	225
PCA1CU	Phragmites	Carex	No	54	107	120	130	148
PCA2	Phragmites	Carex	Yes	53	89	112	142	170
PCA2CU	Phragmites	Carex	No	50	110	116	144	165
PCA3	Phragmites	Carex	Yes	77	108	131	160	173
PCA3CU	Phragmites	Carex	No	76	129	149	174	175
PCA4	Phragmites	Carex	Yes	85	159	200	210	220
PCA4CU	Phragmites	Carex	No	87	130	144	147	147

ID	Phytometer	Neighbour	Competition	10-May-17	27-May-17	07-Jun-17	16-Jun-17	28-Jun-17
PCA5	Phragmites	Carex	Yes	68	113	135	152	180
PCA5CU	Phragmites	Carex	No	70	137	154	187	188
PCA6	Phragmites	Carex	Yes	58	117	134	159	162
PCA6CU	Phragmites	Carex	No	60	106	112	134	135
PCC1	Phragmites	Calamagrostis	Yes	52	137	167	210	210
PCC1CU	Phragmites	Calamagrostis	No	49	108	125	147	150
PCC2	Phragmites	Calamagrostis	Yes	51	126	163	205	219
PCC2CU	Phragmites	Calamagrostis	No	54	140	180	217	240
PCC3	Phragmites	Calamagrostis	Yes	51	132	165	199	215
PCC3CU	Phragmites	Calamagrostis	No	50	79	100	124	230
PCC4	Phragmites	Calamagrostis	Yes	59	155	207	260	292
PCC4CU	Phragmites	Calamagrostis	No	57	136	176	210	220
PCC5	Phragmites	Calamagrostis	Yes	54	106	115	164	170
PCC5CU	Phragmites	Calamagrostis	No	55	113	150	174	185
PCC6	Phragmites	Calamagrostis	Yes	76	118	121	122	125
PCC6CU	Phragmites	Calamagrostis	No	78	129	148	166	180
PTY1	Phragmites	Typha	Yes	63	118	153	190	204
PTY1CU	Phragmites	Typha	No	64	133	165	194	209
PTY2	Phragmites	Typha	Yes	58	133	171	220	236
PTY2CU	Phragmites	Typha	No	56	129	165	170	210
PTY3	Phragmites	Typha	Yes	71	151	183	216	244
PTY3CU	Phragmites	Typha	No	70	134	156	196	222
PTY4	Phragmites	Typha	Yes	80	161	163	235	268
PTY4CU	Phragmites	Typha	No	84	163	198	233	264
PTY5	Phragmites	Typha	Yes	77	132	163	189	211
PTY5CU	Phragmites	Typha	No	75	144	NC	NC	NC
PTY6	Phragmites	Typha	Yes	86	156	201	222	244
PTY6CU	Phragmites	Typha	No	88	146	175	220	238
TYP1	Typha	Phragmites	Yes	85	182	223	252	252
TYP1CU	Typha	Phragmites	No	84	153	194	230	248

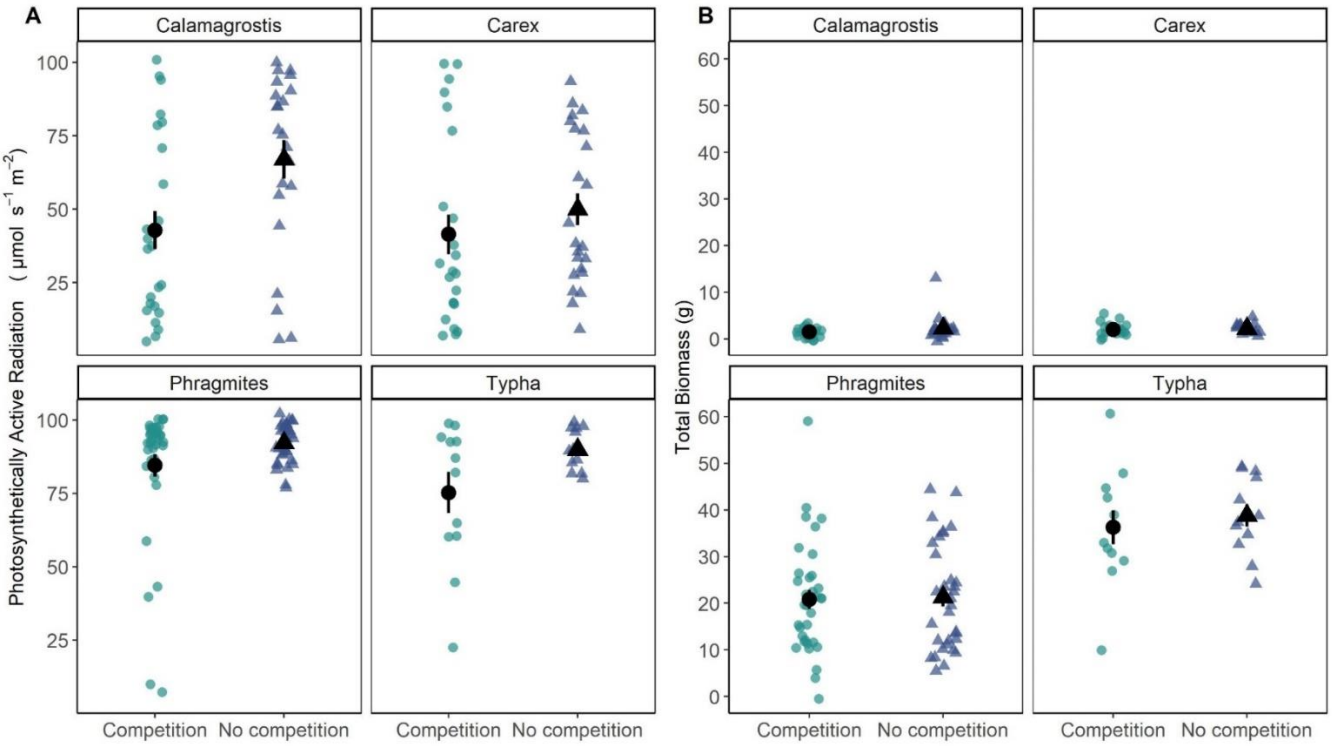
ID	Phytometer	Neighbour	Competition	10-May-17	27-May-17	07-Jun-17	16-Jun-17	28-Jun-17
TYP2	Typha	Phragmites	Yes	76	150	200	255	263
TYP2CU	Typha	Phragmites	No	74	158	102	244	270
TYP3	Typha	Phragmites	Yes	84	170	207	252	288
TYP3CU	Typha	Phragmites	No	82	167	192	229	268
TYP4	Typha	Phragmites	Yes	87	173	118	267	284
TYP4CU	Typha	Phragmites	No	89	174	217	247	274
TYP5	Typha	Phragmites	Yes	76	179	218	276	302
TYP5CU	Typha	Phragmites	No	76	144	186	210	244
TYP6	Typha	Phragmites	Yes	70	162	187	249	288
TYP6CU	Typha	Phragmites	No	72	157	190	245	270



Appendix 1D. Height of phytometers at establishment in 2016 (n = 96) and 2017 (n = 96). Phytometers were paired by height to account for natural variation among plants in the field.



Appendix 1E. Illustration of the experimental design. Phytometers represent one individuals of a representative species growing either without competition (1 m^2 area around phytometer cleared) or with competition. Resident species phytometers grew surrounded by *P. australis* while *P. australis* phytometers grew surrounded by *C. aquatilis*, *C. calamagrostis,* or *Typha* spp. As all species are clonal, we severed to a 50 cm depth around the 1 m^2 perimeter of each plot



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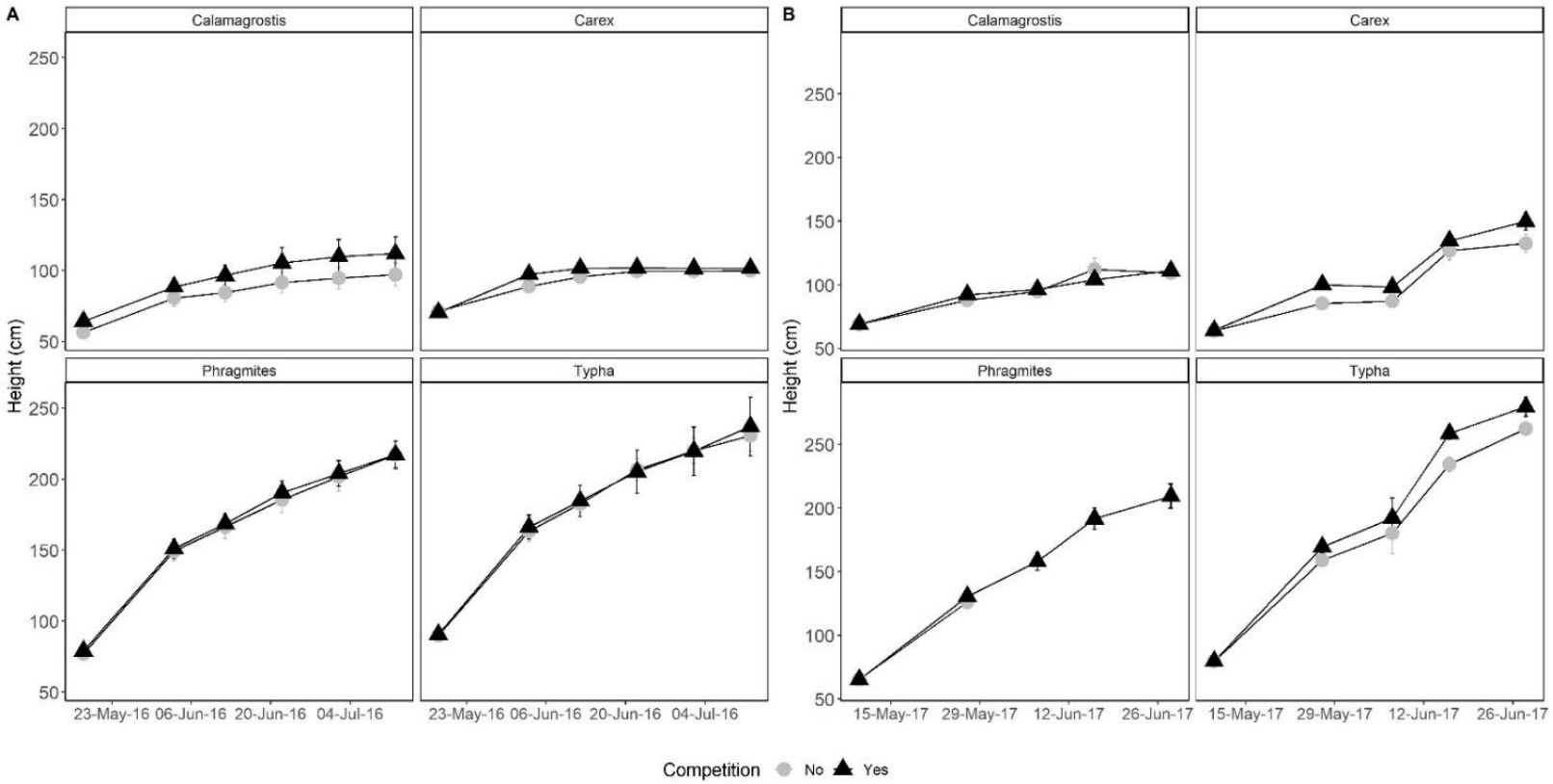
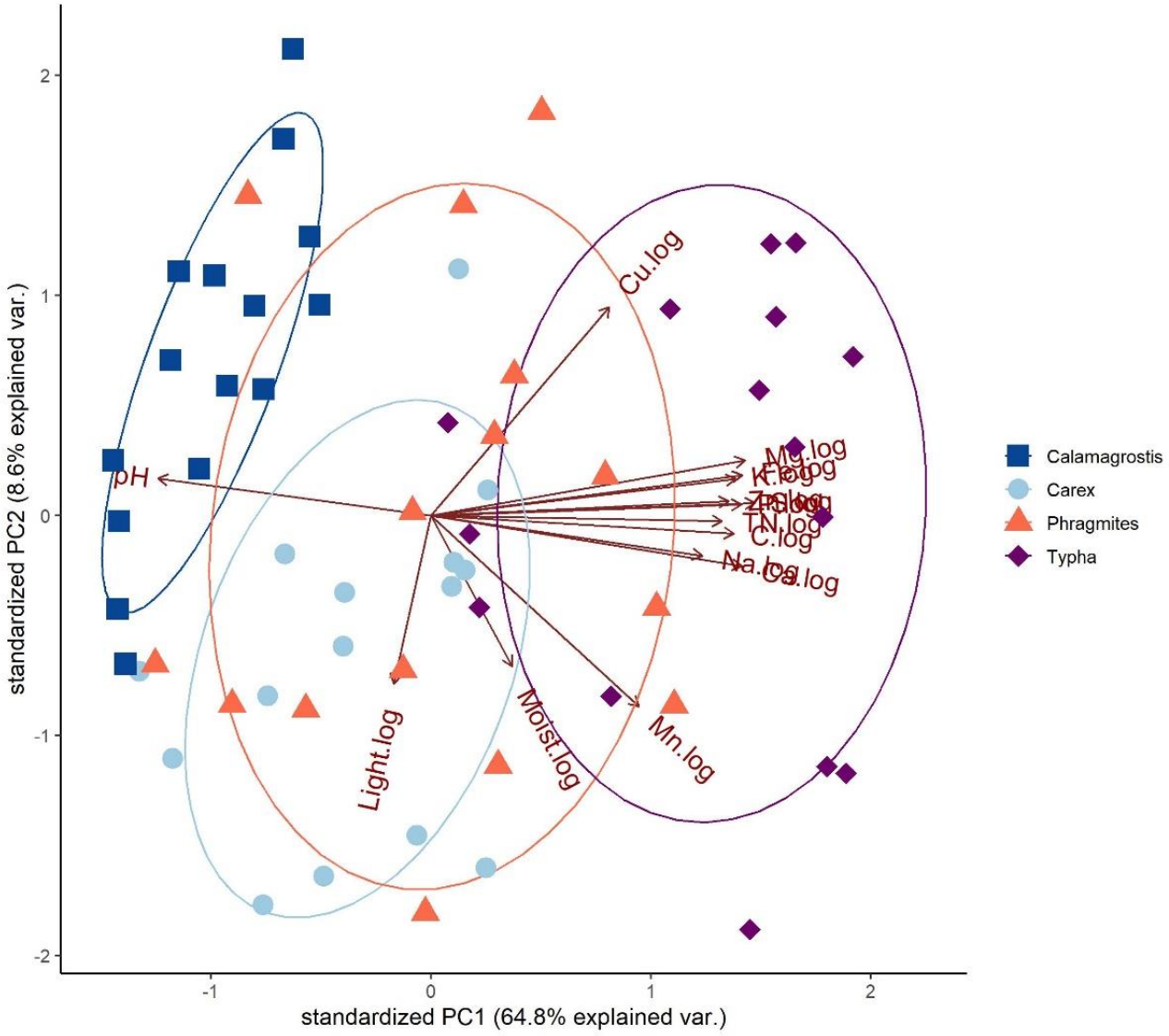
22 Appendix 1F. The percent of photosynthetically active radiation reaching the top of each
23 phytometer growing with or without competition (A) and the total aboveground biomass
24 produced by each species growing with or without competition (B). Large symbols represent the
25 average and error bars represent standard error.



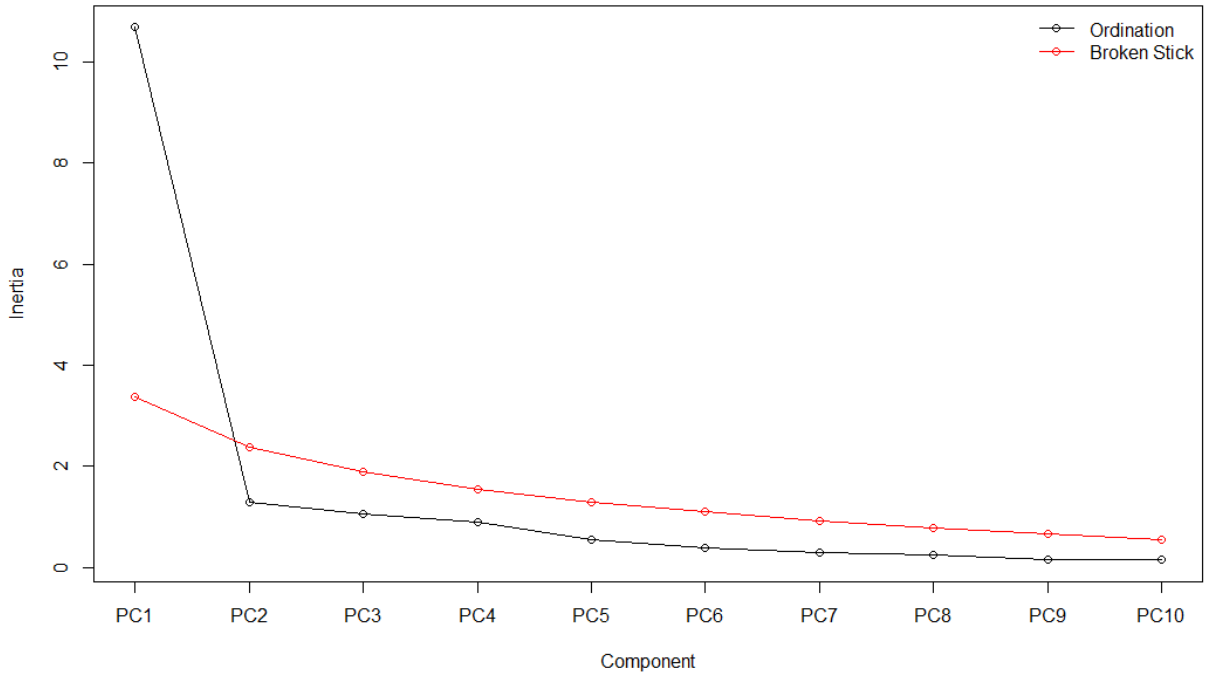
Appendix 1G. The growth of phytometers over the 2016 (A) and 2017 (B) growing seasons. Resident species represent remnant emergent marsh (*Typha* spp.), and meadow marsh (*Carex aquatilis, Calamagrostis canadensis*) growing with or without above-ground competition with *P. australis*, while invasive *P. australis* grew with or without above-ground competition from resident species. Error bars represent standard error.



Appendix 1H. A principal components analysis using measured soil nutrients and percent incident light from marsh vegetation communities dominated by the target species: invasive *P. australis*, *C. canadensis* and *C. aquatilis* which represent remnant meadow marsh, and *Typha* spp. which represents remnant emergent marsh. The first two axes explain 75% of the variance in the data (Axis 1: 0.667, Axis 2: 0.080) and were used to assess niche overlap among species. Ellipses are normal data ellipses for each group, vectors represent all variables included in the PCA (Appendix 1I).

Appendix 1I. Eigenvalue and variance explained by the first three axes of our PCA, and the eigenvector values of all environmental variables included in the analysis.

	PC1	PC2
Eigenvalue	9.720	1.286
Proportion explained	0.648	0.086
Soil moisture (%)	0.34293	-0.63565
Copper (Cu)	0.753489	0.879554
Iron (Fe)	1.313269	0.168952
Manganese (Mn)	0.877403	-0.80537
Phosphorus (P)	1.310303	0.045938
Magnesium (Mg)	1.32475	0.231848
Potassium (K)	1.28684	0.154487
Zinc (Zn)	1.256093	0.059001
Carbon (C)	1.277762	-0.07677
Total Nitrogen (TN)	1.228042	-0.02341
Sodium (Na)	1.144614	-0.1705
Calcium (Ca)	1.312584	-0.21473
Sulphur (S)	1.365828	0.052466
Incident light (%)	-0.15694	-0.71027
Soil pH	-1.1525	0.155776



Appendix 1J. The scree plot assessing inertia of the environmental variable PCA. Axis 1 and 2 were selected for further analyses.

Appendix 2A. Water depths from every control and treatment plot in Long Point (LP) and Rondeau Provincial Park (RPP). Control (n = 39) and treatment (n = 41) plots were paired by August 2016 water depths across the range occupied by invasive *P. australis* at sufficient density to warrant herbicide application (> 20 stem m^{-2}). Control plot RCP16 was mistakenly treated and thus was re-coded. Further, control plot RPC41 became inaccessible in 2018, reducing the number of control plots to 38.

SiteID	Location	Treatment	Water Depth (cm)		
			2016	2017	2018
LPC25	LP	Control	10	38	27
LPC24	LP	Control	13	36	44
LPC39	LP	Control	13	28	20
RPC04	RPP	Control	13	29	37
RPC15	RPP	Control	13	14.5	34
LPC26	LP	Control	14	39	52
LPC27	LP	Control	17	42	50
RPC14	RPP	Control	17	36	56
LPC33	LP	Control	18	32	34
LPC37	LP	Control	18	46	40
LPC32	LP	Control	19	40	46
LPC31	LP	Control	20	46	36
RPC05	RPP	Control	20	37	50
RPC08	RPP	Control	20	45	46
RPC18	RPP	Control	20	39	53
LPC21	LP	Control	21	52	60
RPC12	RPP	Control	21	42	50
LPC28	LP	Control	23	44	54
RPC11	RPP	Control	23	46	43
RPC20	RPP	Control	23	40	43
LPC23	LP	Control	24	59	52
LPC36	LP	Control	24	45	45
RPC10	RPP	Control	24	41	48.5
RPC07	RPP	Control	25	46	53.5
LPC30	LP	Control	26	49	41
LPC35	LP	Control	28	54	43
LPC40	LP	Control	28	33	35
LPC29	LP	Control	29	53	61
RPC03	RPP	Control	32	49	60.5
RPC06	RPP	Control	32	42	62
RPC09	RPP	Control	32	37	53
RPC17	RPP	Control	32	49.5	61
LPC38	LP	Control	34	53	35

SiteID	Location	Treatment	Water Depth (cm)		
			2016	2017	2018
RPC13	RPP	Control	34	42	42
LPC22	LP	Control	36	48	63
LPC34	LP	Control	38	61	44
RPC02	RPP	Control	42	53.5	54
RPC19	RPP	Control	44	48	55
RPC41	RPP	Control	48	79	NC
LPT13	LP	Treatment	13	43	39
LPT41	LP	Treatment	13	43	32
RPT28	RPP	Treatment	16	35	39
RPT34	RPP	Treatment	16	28.5	45
LPT15	LP	Treatment	17	44.5	35
RPT32	RPP	Treatment	17	39	34
LPT12	LP	Treatment	18	46.5	39
LPT19	LP	Treatment	18	52	62
RPT22	RPP	Treatment	18	52	53
RPT37	RPP	Treatment	18	41	47
LPT16	LP	Treatment	19	42	31
RPT31	RPP	Treatment	20	38	45
LPT08	LP	Treatment	21	45	40
LPT18	LP	Treatment	21	55	40
RPT25	RPP	Treatment	21	45	53
RPT39	RPP	Treatment	21	42.5	45
LPT07	LP	Treatment	22	43.5	44
LPT10	LP	Treatment	22	60	41
LPT14	LP	Treatment	22	43.5	43
RPT30	RPP	Treatment	22	46	58
LPT09	LP	Treatment	24	49	34
LPT20	LP	Treatment	24	43	38
LPT42	LP	Treatment	24	55	47
RPT40	RPP	Treatment	24	44	46
LPT43	LP	Treatment	25	50	39
RPT24	RPP	Treatment	26	49	58
LPT11	LP	Treatment	28	58	44
LPT06	LP	Treatment	30	43	43
LPT05	LP	Treatment	31	65	43
RPT23	RPP	Treatment	31	55	61
RPT33	RPP	Treatment	31	46	57
RPT29	RPP	Treatment	32	45	40
LPT44	LP	Treatment	33	61.5	29
RPT21	RPP	Treatment	33	62	64
RPT38	RPP	Treatment	33	48	61
RPC16	RPP	Treatment	41	25	48

SiteID	Location	Treatment	Water Depth (cm)		
			2016	2017	2018
LPT17	LP	Treatment	42	62	5
RPT27	RPP	Treatment	44	57.5	65
RPT36	RPP	Treatment	45	44	92
RPT35	RPP	Treatment	46	48	61
RPT26	RPP	Treatment	47	65.5	86

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Appendix 2B. Two-way ANOVA results, type III SS with year (2016, 2017, 2018) and treatment as fixed factors. The percent of PAR penetrating the canopy was log_{10} transformed. There was a significant interaction for every measured variable.

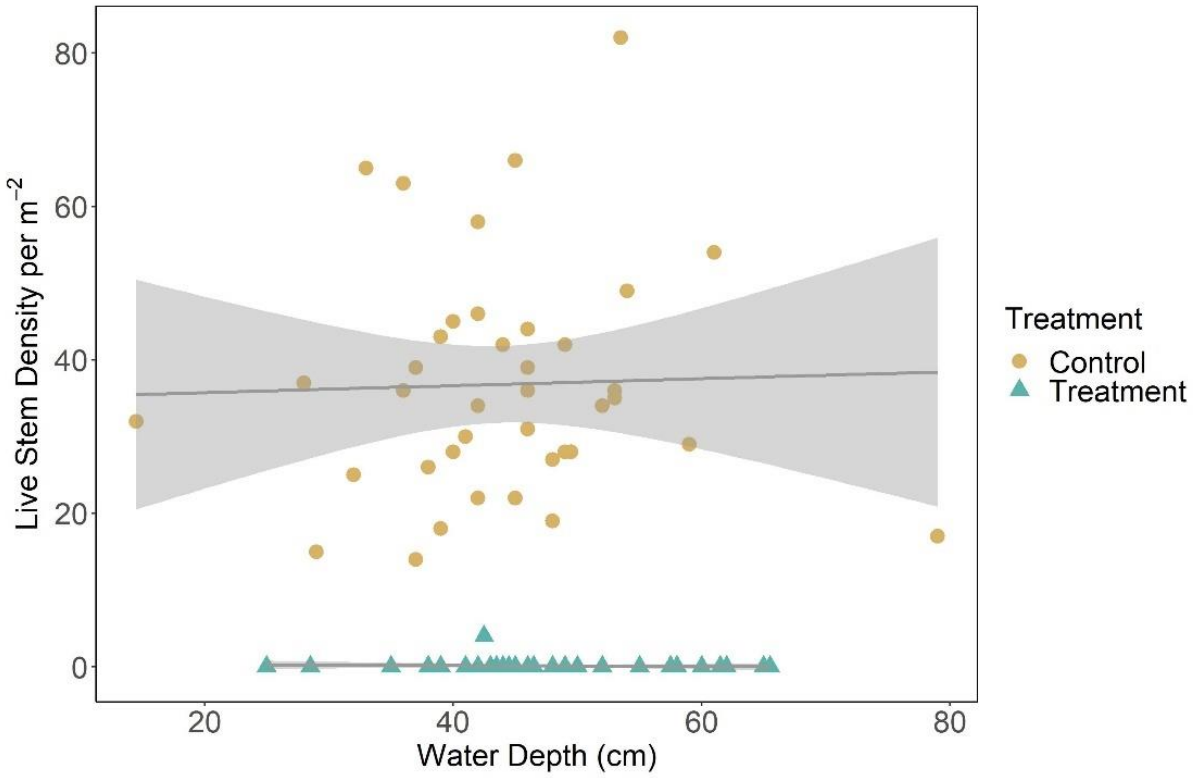
	Live stems (per m^2)			Total stems (per m^2)			Canopy Height (cm)			PAR penetration (% incident light)		
	df	F	P	df	F	P	df	F	P	df	F	P
Year	2	3.615	0.028	2	0.448	0.639	2	0.267	0.766	2	1.697	0.186
Treatment	1	0.735	0.392	1	0.312	0.577	1	0.047	0.828	1	1.243	0.266
Year x Treatment	2	60.612	< 0.001	2	19.293	< 0.001	2	138.250	< 0.001	2	51.208	< 0.001
Error	233			233			233			233		

Appendix 2C. The average, standard deviation, minimum and maximum live invasive *P. australis* stem density, total (live & dead) stem density, canopy height and photosynthetically active radiation (PAR) penetration in both treatments over the three years; pre-treatment (2016) and two years post-treatment (2017 and 2018).

		Live Stems (per m^{-2})			Total Stems (per m^{-2})			Canopy Height (cm)			PAR penetration (% incident light)		
		Avg. (std.)	Min.	Max.	Avg. (std.)	Min.	Max.	Avg. (std.)	Min.	Max.	Avg. (std.)	Min.	Max.
Control	2016	34.7 (13.1)	13	68	102 (46.2)	20	193	362 (61.1)	237	526	4.1 (4.3)	0.4	17.0
	2017	36.8 (15.3)	14	82	112 (49.0)	15	229	372 (50.4)	270	470	6.0 (7.5)	0.6	33.8
	2018	29.8 (12.3)	10	66	105 (49.5)	37	200	367 (47.4)	260	460	6.0 (8.6)	0.7	50.6
Treatment	2016	37.0 (15.8)	11	81	108 (47.5)	23	229	358 (41.5)	285	470	4.7 (3.9)	0.3	13.6
	2017	0.1 (0.6)	0	4	50.2 (52.3)	0	120	40.7 (65.5)	0	207	56.3 (27.4)	11.0	99.6
	2018	1.5 (5.6)	0	28	24.3 (30.5)	0	198	121 (102)	0	275	61.1 (26.6)	9.0	99.4

Appendix 2D. Two-way ANOVA results, type III SS, comparing total stem density, live stem density, and photosynthetically active radiation (PAR) penetration in 2017 and 2018 between Long Point and Rondeau Provincial Park. Secondary treatment (e.g., rolling and mowing) occurred in Long Point but not Rondeau. The percent of PAR penetrating the canopy was $\log_{10}$ transformed.

	Total stems (per m^{-2})			Live stems (per m^{-2})			PAR penetration (% incident light)		
	df	F	P	df	F	P	df	F	P
Year	1	1.258	0.265	1	0.883	0.350	1	2.476	0.120
Location	1	51.908	< 0.001	1	0.023	0.880	1	7.085	0.009
Year x Location	1	4.286	0.0418	1	0.055	0.815	1	8.460	0.005
Error	78			78			78		



Appendix 2E. Glyphosate-based herbicide was effective at suppressing invasive *P. australis* along the entire water depth gradient. The number of live invasive *P. australis* stems (per m^2) in the control plots one year after treatment occurred were significantly higher than in the herbicide-treated plots and there was no difference in live invasive *P. australis* stem density along the water depth gradient (cm). Shaded area represents 95% confidence intervals. Made with ggplot2 (Whickham 2016).

Appendix 2F. A significant difference in vegetation community composition arose between treatment and control plots after the herbicide was applied (i.e., the interaction term was significant). Thus, we conclude that treatment had an effect on the vegetation community structure. Results represent the average test statistics taken from 500 runs of a perMANOVA performed using stratified random sampling with replacement. Values in parentheses are the standard deviation.

	df	Average Pseudo-F	Average p-value
Treatment	1	170.20 (± 14.90)	0.001 (< 0.001)
Year	2	61.51 (± 6.13)	0.001 (< 0.001)
Treatment x year	2	44.77 (± 5.33)	0.001 (< 0.001)
Residual	222		

Appendix 2G. The correlation coefficients (r) and coefficient of determination (r^2) of all the vectors in the 3D NMDS ordination for the control, treatment, and reference condition plots. Vectors with an $r^2 \geq 0.150$ are considered reasonably correlated with points and were included in Figure 4.

		Axis 1		Axis 2		Axis 3	
Full name	Taxonomic Authority	r	r^2	r	r^2	r	r^2
Water depth		-0.271	0.073	0.215	0.046	0.144	0.021
Open water		-0.685	0.469	-0.507	0.257	-0.141	0.020
Litter		-0.012	0.000	0.055	0.003	-0.549	0.301
Standing dead		-0.438	0.192	-0.264	0.070	0.529	0.280
Calamagrostis canadensis	(Michx.) P. Beauv.	-0.145	0.021	-0.330	0.109	-0.249	0.062
Campanula aparinoides	Pursh	-0.037	0.001	-0.105	0.011	-0.042	0.002
Calystegia sepium	(L.) R. Br.	-0.070	0.005	-0.090	0.008	0.055	0.003
Carex aquatilis	Wahlenb.	-0.050	0.003	-0.169	0.029	-0.220	0.048
Carex buxbaumii	Wahlenb.	-0.149	0.022	-0.157	0.025	-0.298	0.089
Carex crawei	Dewey	-0.102	0.010	-0.128	0.016	-0.058	0.003
Carex comosa	Boott	0.063	0.004	0.014	0.000	-0.010	0.000
Carex lacustris	Willd.	-0.036	0.001	0.026	0.001	0.206	0.043
Carex lasiocarpa	Ehrh.	0.017	0.000	-0.119	0.014	-0.134	0.018
Carex sartwellii	Olney	-0.055	0.003	-0.054	0.003	-0.291	0.085
Carex spp.	L.	0.055	0.003	-0.004	0.000	-0.045	0.002
Cladium mariscoides	(Muhl.) Torr.	-0.130	0.017	-0.109	0.012	-0.392	0.154
Cornus sericea spp. *sericea*	L.	-0.146	0.021	-0.172	0.030	0.005	0.000
Decodon verticillatus	(L.) Elliott	-0.010	0.000	0.058	0.003	-0.009	0.000
Dulichium arundinaceum	(L.) Britton	0.091	0.008	-0.006	0.000	0.007	0.000
Eleocharis palustris	(L.) Roem. & Schult.	0.173	0.030	-0.023	0.001	-0.017	0.000
Elodea canadensis	Michx.	0.041	0.002	-0.056	0.003	-0.010	0.000
Eleocharis spp.	R. Br.	-0.056	0.003	-0.058	0.003	-0.104	0.011
Equisetum fluviatile	L.	-0.071	0.005	0.183	0.034	0.017	0.000
Fontinalis sp.	Hedw.	-0.103	0.011	-0.028	0.001	-0.016	0.000
Galium aparine	L.	0.079	0.006	-0.013	0.000	-0.001	0.000

Full name	Taxonomic Authority	Axis 1		Axis 2		Axis 3	
		r	r^2	r	r^2	r	r^2
Hydrocharis morsus-ranae	L.	-0.384	0.147	0.866	0.749	-0.056	0.003
Hypericum kalmianum	L.	-0.128	0.016	-0.174	0.030	-0.081	0.007
Iris versicolor	L.	-0.101	0.010	-0.127	0.016	-0.103	0.011
Juncus balticus	Willd.	-0.059	0.003	-0.054	0.003	-0.226	0.051
Lemna minor	L.	-0.042	0.002	0.137	0.019	-0.073	0.005
Lysimachia thyrsiflora	L.	-0.145	0.021	-0.156	0.024	-0.156	0.024
Achillea millefolium	L.	-0.132	0.017	-0.016	0.000	-0.096	0.009
Myriophyllum sibiricum	Kom.	-0.082	0.007	0.022	0.000	-0.046	0.002
*Myriophyllum*spp.	L.	-0.088	0.008	0.013	0.000	0.136	0.019
Nuphar variegata	Durand	0.029	0.001	0.037	0.001	-0.094	0.009
Nymphaea odorata	Aiton	0.006	0.000	0.148	0.022	-0.031	0.001
Phragmites australis ssp. *australis*	(Trin.) ex	0.968	0.937	-0.078	0.006	0.080	0.006
Persicaria amphibia	(L.) Delarbre	0.102	0.011	-0.017	0.000	0.071	0.005
*Polygonum*spp.	(L.) Mill.	-0.133	0.018	-0.071	0.005	0.198	0.039
Potamogeton spp.	L.	-0.083	0.007	-0.067	0.004	-0.058	0.003
Sagittaria spp.	L.	0.054	0.003	-0.028	0.001	0.026	0.001
Sagittaria latifolia	Willd.	-0.068	0.005	0.112	0.013	-0.057	0.003
Schoenoplectus acutus var. *acutus*	(Muhl. Ex Bigelow) A. Love & D. Love	-0.028	0.001	-0.079	0.006	-0.007	0.000
Bolboschoenus fluviatilis	(Torr.) Sojak	0.074	0.006	0.004	0.000	-0.047	0.002
Schoenoplectus tabernaemontani	(C.C. Gmel.) Palla	0.046	0.002	0.010	0.000	-0.004	0.000
Sparganium eurycarpum	Engelm.	-0.072	0.005	0.209	0.044	0.026	0.001
Sparganium spp.	L.	-0.075	0.006	0.005	0.000	-0.052	0.003
Spirodela polyrrhiza	(L.) Schleid.	0.065	0.004	0.010	0.000	0.005	0.000
Solanum spp.	L.	0.046	0.002	0.030	0.001	-0.027	0.001
Solidago spp.	L.	-0.113	0.013	-0.143	0.020	-0.051	0.003
Typha spp.	L.	-0.313	0.098	0.048	0.002	0.595	0.354
Typha angustifolia	L.	0.220	0.048	-0.016	0.000	-0.125	0.016
Typha x *glauca*	Godr. (pro sp.)	0.180	0.032	0.065	0.004	-0.231	0.053
Typha latifolia	L.	0.060	0.004	-0.022	0.001	-0.049	0.002

		Axis 1		Axis 2		Axis 3	
Full name	Taxonomic Authority	r	r^2	r	r^2	r	r^2
Utricularia intermedia	Hayne	-0.111	0.012	0.004	0.000	-0.222	0.049
Utricularia vulgaris	L.	-0.157	0.025	-0.052	0.003	-0.045	0.002
Unknown		-0.093	0.009	-0.069	0.005	-0.083	0.007
Zizania palustris	L.	-0.085	0.007	-0.115	0.013	-0.033	0.001

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Appendix 3A. Study sites used to evaluate aquatic and emerging invertebrate communities in Long Point, ON. Sites were established in early May 2018. There were collections approximately every 10 days in 2018 which resulted in a total of six collections over the field season. Collection bottles were not always successfully retrieved, typically due to weather, and the number of collections per site are noted as is water depth in May 2018.

Site	Treatment	Year	Habitat	Depth (cm)	Number of collections
IN1	Invaded	2018	Phragmites	23.3	6
IN2	Invaded	2018	Phragmites	58.0	6
IN3	Invaded	2018	Phragmites	22.3	6
IN4	Invaded	2018	Phragmites	28.7	6
IN5	Invaded	2018	Phragmites	42.0	6
IN6	Invaded	2018	Phragmites	26.3	6
IN7	Invaded	2018	Phragmites	70.5	6
IN8	Invaded	2018	Phragmites	48.3	6
IN9	Invaded	2018	Phragmites	77.0	5
RES1	Treated	2018	Herbicide-treated	26.7	6
RES2	Treated	2018	Herbicide-treated	27.2	6
RES3	Treated	2018	Herbicide-treated	29.7	6
RES4	Treated	2018	Herbicide-treated	35.3	5
RES5	Treated	2018	Herbicide-treated	21.7	4
RES6	Treated	2018	Herbicide-treated	51.0	6
RES7	Treated	2018	Herbicide-treated	24.0	6
RES8	Treated	2018	Herbicide-treated	23.2	6
RES9	Treated	2018	Herbicide-treated	49.7	5
UNM1	Uninvaded	2018	Meadow	16.3	6
UNM2	Uninvaded	2018	Meadow	16.7	6
UNM3	Uninvaded	2018	Meadow	7.0	6
UNM4	Uninvaded	2018	Meadow	15.0	6
UNM5	Uninvaded	2018	Meadow/mixed	24.7	6
UNM6	Uninvaded	2018	Meadow/mixed	32.8	6
UNT9	Uninvaded	2018	Emergent	75.0	5
UNT1	Uninvaded	2018	Emergent	34.8	6

Site	Treatment	Year	Habitat	Depth (cm)	Number of collections
UNT2	Uninvaded	2018	Emergent	36.2	6

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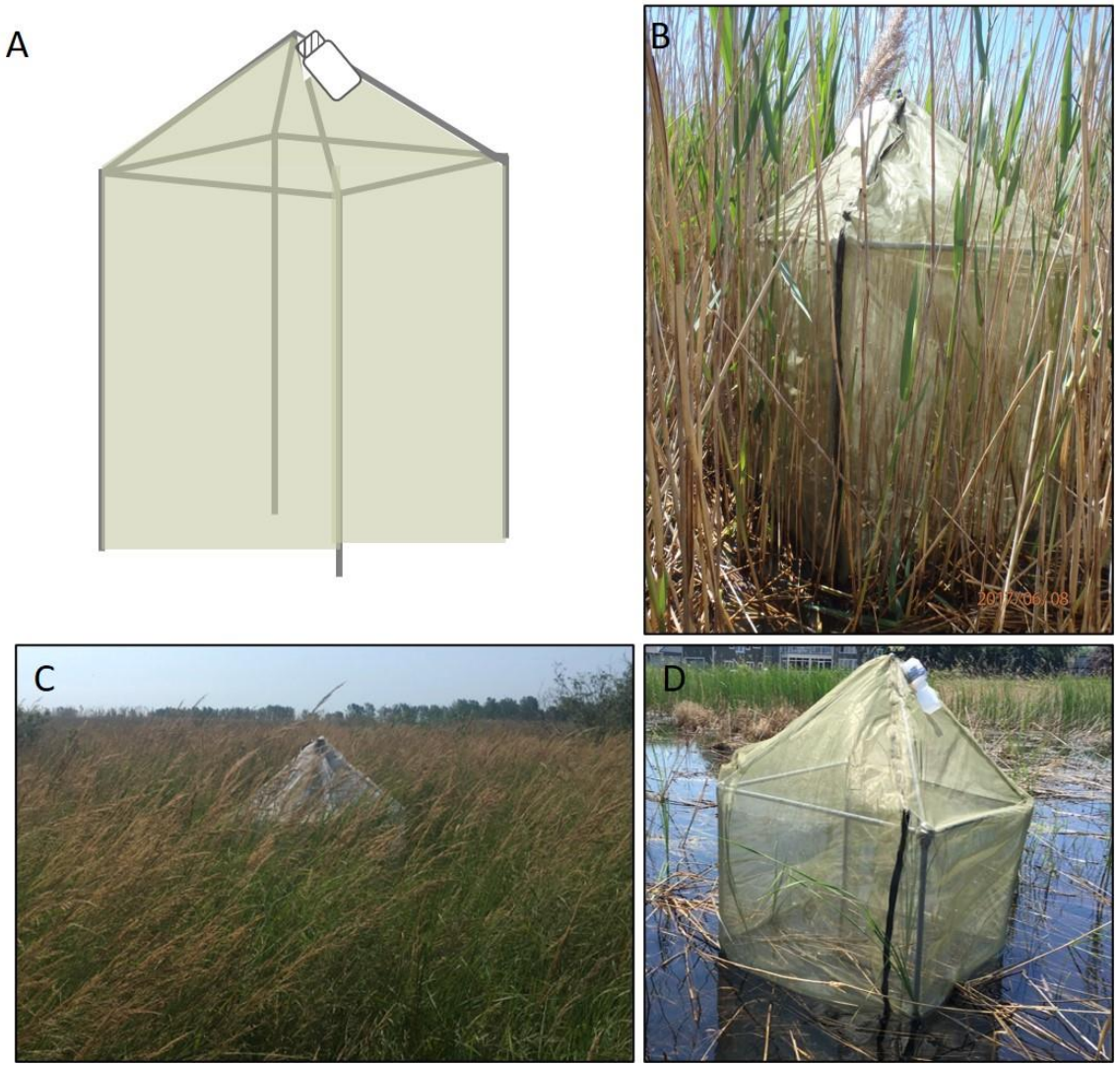

Appendix 3B. Emergence trap schematic (A), and placed in *P. australis* (B), meadow marsh (C), and herbicide-treated sites (D) in Long Point, ON.

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98 Appendix 3C. Emerging invertebrate taxa identified in emergence trap (1 m^2) samples collected from herbicide-treated, *P. australis,*
99 and remnant marsh sites from Long Point, ON from May to July 2018.

Taxa	Taxonomic level	Order
Agromyzidae	Subfamily	Diptera
Anthicidae	Family	Coleoptera
Anthicidae larvae		
Anthocoridae	Family	Hemiptera
Anthomyiidae	Family	Diptera
Aphelinidae	Family	Hymenoptera
Aphididae	Family	Hemiptera
Araneae	Order	
Braconidae	Family	Hymenoptera
Caenidae	Family	Ephemeroptera
Calliphoridae	Family	Diptera
Carabidae	Family	Coleoptera
Carnidae	Family	Diptera
Cecidomyiidae	Family	Diptera
Ceraphronidae	Family	Hymenoptera
Ceratopogonidae	Family	Diptera
Chaoboridae	Family	Diptera
Chironomidae	Family	Diptera
Chloropidae	Family	Diptera
Cicadellidae	Family	Hemiptera
Coccinellidae Larvae		
Coccinellidae	Family	Coleoptera
Coenagrionidae	Family	Odonata
Collembola	Order	
Cosmopterigidae	Family	Lepidoptera
Crambidae	Family	Lepidoptera

Taxa	Taxonomic level	Order
Cryptophagidae	Family	Coleoptera
Culicidae	Family	Diptera
Curculionidae	Family	Coleoptera
Dixidae	Family	Diptera
Dolichopodidae	Family	Diptera
Drosophilidae	Family	Diptera
Empididae	Family	Diptera
Encyrtidae	Family	Hymenoptera
Ephemerellidae	Family	Ephemeroptera
Ephydridae	Family	Diptera
Erotylidae larvae		
Erotylidae	Family	Coleoptera
Eulophidae	Family	Hymenoptera
Eurytomidae	Family	Hymenoptera
Formicidae	Family	Hymenoptera
Halictidae	Family	Hymenoptera
Hydroptilidae	Family	Trichoptera
Ichneumonidae	Family	Hymenoptera
Lampyridae	Family	Coleoptera
Latridiidae	Family	Coleoptera
Leptoceridae	Family	Trichoptera
Lestidae	Family	Odonata
Libellulidae	Family	Odonata
Limnephilidae	Family	Trichoptera
Megaspilidae	Family	Hymenoptera
Miridae	Family	Hemiptera
Muscidae	Family	Diptera
Mymaridae	Family	Hymenoptera
Nitidulidae	Family	Coleoptera
Noctuidae	Family	Lepidoptera

Taxa	Taxonomic level	Order
Phalacridae	Family	Coleoptera
Phoridae	Family	Diptera
Phryganeidae	Family	Trichoptera
Platygastridae	Family	Hymenoptera
Polycentropodidae	Family	Trichoptera
Pompilidae	Family	Hymenoptera
Psocoptera	Order	
Pteromalidae	Family	Hymenoptera
Scelionidae	Family	Hymenoptera
Sciaridae	Family	Diptera
Sciomyzidae	Family	Diptera
Staphylinidae	Family	Coleoptera
Stratiomyidae	Family	Diptera
Syrphidae	Family	Diptera
Tabanidae	Family	Diptera
Thysanoptera	Order	
Tipulidae	Family	Diptera

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Appendix 3D. Aquatic invertebrate taxa identified in the ¼ m^2 vegetation quadrats collected from herbicide-treated, *P. australis,* and remnant marsh sites from Long Point, ON in May 2018.

Taxa	Taxonomic level	Order
Arachnida	Subclass	
Amphipoda	Order	
Araneae	Order	
Bivalvia	Class	
Caenidae	Family	Ephemeroptera
Cecidomyiidae	Family	Diptera
Ceratopogonidae	Family	Diptera
Chironomidae	Family	Diptera
Coenagrionidae	Family	Odonata
Collembola	Order	
Corduliidae	Family	Odonata
Crambidae	Family	Lepidoptera
Culicidae	Family	Diptera
Curculionidae	Family	Coleoptera
Brachycera	Suborder	
Dolichopodidae	Family	Diptera
Dytiscidae	Family	Coleoptera
Ephydridae	Family	Diptera
Gastropoda	Class	
Lumbriculata	Subclass	
Hydrophilidae	Family	Coleoptera
Hydrozoa	Class	
Isopoda	Order	
Lampyridae	Family	Coleoptera
Leptoceridae	Family	Trichoptera
Limnephilidae	Family	Trichoptera

Nematoda	Phylum	
Oligochaeta	Subclass	
Ostracoda	Class	
Platyhelminthes	Phylum	
Pleidae	Family	Hemiptera
Sciomyzidae	Family	Diptera
Scirtidae	Family	Coleoptera
Staphylinidae	Family	Coleoptera
Stratiomyidae	Family	Diptera
Thysanoptera	Order	
Tipulidae	Family	Diptera

Appendix 3E. Taxa included in Procrustes test analysis from the emergence trap samples (emerging invertebrate samples) and the aquatic invertebrate samples (aquatic invertebrate samples). Taxa were identified to lowest feasible taxonomic level.

Taxa	Samples
Caenidae	Emergence trap
Cecidomyiidae	Emergence trap
Ceratopogonidae	Emergence trap
Chironomidae	Emergence trap
Coenagrionidae	Emergence trap
Crambidae	Emergence trap
Culicidae	Emergence trap
Dolichopodidae	Emergence trap
Ephydridae	Emergence trap
Lampyridae	Emergence trap
Leptoceridae	Emergence trap
Limnephilidae	Emergence trap
Sciomyzidae	Emergence trap
Stratiomyidae	Emergence trap
Tabanidae	Emergence trap

Taxa	Samples
Tipulidae	Emergence trap
Dixidae	Emergence trap
Hydroptilidae	Emergence trap
Lestidae	Emergence trap
Libellulidae	Emergence trap
Phryganeidae	Emergence trap
Polycentropodidae	Emergence trap
Chaoboridae	Emergence trap
Syrphidae	Emergence trap
Ichneumonidae	Emergence trap
Scelionidae	Emergence trap
Mymaridae	Emergence trap
Eulophidae	Emergence trap
Pteromalidae	Emergence trap
Phoridae	Emergence trap
Cosmopterigidae	Emergence trap
Caenidae	Aquatic sample
Cecidomyiidae	Aquatic sample
Ceratopogonidae	Aquatic sample
Chironomidae	Aquatic sample
Coenagrionidae	Aquatic sample
Crambidae	Aquatic sample
Culicidae	Aquatic sample
Dolichopodidae	Aquatic sample
Ephydridae	Aquatic sample
Lampyridae	Aquatic sample
Leptoceridae	Aquatic sample
Limnephilidae	Aquatic sample
Sciomyzidae	Aquatic sample

Taxa	Samples
Stratiomyidae	Aquatic sample
Tipulidae	Aquatic sample
Tabanidae	Aquatic sample
Corduliidae	Aquatic sample

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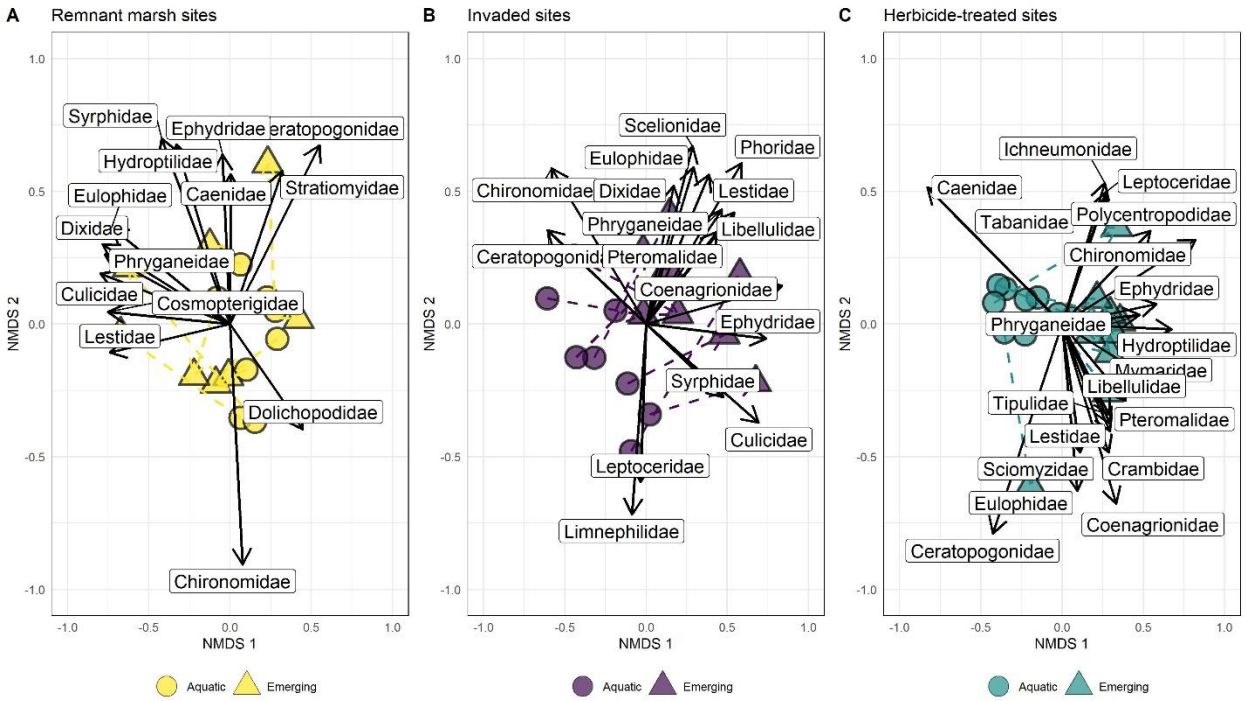

Appendix 3F. NMDS ordination of emerging and aquatic invertebrate communities present in each habitat.). The uninvaded NMDS had a 2D final solution with a stress of 0.161 after 88 iterations and a non-metric r^2 of 0.974 (A), the invaded emerging invertebrate NMDS had a 2D final solution with a stress of 0.138 after 48 iterations and a non-metric r^2 of 0.981 (B), and the herbicide-treated sites had a 2D final solution with a stress of 0.080 after 49 iterations and a non-metric r^2 of 0.993 (C). Aquatic invertebrates were collected in mid-May 2018 from submersed aquatic vegetation and emerging invertebrates were collected from 05-June-18 to 23-July-18. Reasonably correlated taxa ($r^2 > 0.30$) are included as vectors and ellipses are 90% CI.